National Institute on Alcohol Abuse and Alcoholism
ADAMHA

Annual Report of Intramural Research

October 1, 1989 to September 30, 1990

NUMERICAL INVENTORY OF PROJECTS

DIVISION OF INTRAMURAL CLINICAL AND BIOLOGICAL RESEARCH

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

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Z01 AA 00036-04 LMMB B.J. Song	Regulation of ethanol-inducible cytochrome P450 gene (III)
Z01 AA 00037-04 LMMB B.J. Song	Structure and regulation of genes for pyruvate dehydrogenase multienzyme complex (III)
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Z01 AA 00039-03 LMMB A.C. McLaughlin	Cerebral blood flow and energy metabolism in the rat
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Z01 AA 00231-08 LCS M.J. Eckardt	Central and peripheral nervous system function in abstinent alcoholics
Z01 AA 00233-08 LCS G. Brown	Family studies of alcoholism
Z01 AA 00234-08 LCS D. Goldman	Molecular genetic studies of alcoholism
Z01 AA 00235-09 LCS N. Salem, Jr.	Nutritional effects on lipid composition
Z01 AA 00237-08 LCS S. Shoaf	Individual variability in drug metabolism by carbon dioxide breath tests
Z01 AA 00238-08 LCS M. Linnoila	CSF neuropeptides and prostaglandins in alcohol withdrawal and brain disease
Z01 AA 00239-07 LCS M. Eckardt	Alcoholism-assoicated cognitive impairment and organic brain syndrome
Z01 AA 00240-11 LCS M. Eckardt	Cognitive function in male alcoholics
Z01 AA 00249-07 LCS D. George	Pharmacologic reduction of alcohol consumption in alcoholic patients
Z01 AA 00250-07 LCS J. Rohrbaugh	Electrophysiological studies of acute and chronic alcohol consumption
Z01 AA 00257-06 LCS G. Brown	Neuroendocrine studies in offspring of familial alcoholics
Z01 AA 00258-06 LCS M. Linnoila	Violent behavior, neurotransmitters, glucose metabolism, and alcohol abuse
Z01 AA 00262-07 LCS N. Salem	Characterization of fatty acid metabolites by GC/MC

Z01 AA 00265-05 LCS D. George	Effects of alprazolam, diazepam, clonidine, and placebo upon ethanol withdrawal
Z01 AA 00266-05 LCS D. George	Relationship of psychopathology to neurofunction in alcoholics
Z01 AA 00267-05 LCS M. Eckardt	Brain imaging
Z01 AA 00268-05 LCS R. Lister	The behavioral effects of alcohol and other psychotropic drugs
Z01 AA 00270-05 LCS A. Roy	Impulsivity and pathologic gambling
Z01 AA 00272-03 LCS M. Linnoila	CSF monoamine metabolites in alcoholic patients who attempt suicide
Z01 AA 00273-02 LCS D. George	Effects of serotonergic activity on neuroendocrine and behavioral measures
Z01 AA 00274-02 LCS D. George	Intravenous procaine in alcoholics and adult children of alcoholics
Z01 AA 00275-021 LCS M. Eckardt	Psychomotor and cognitive aspects of alcoholism
Z01 AA 00276-02 LCS G. Brown	Psychobiology and behavior of aggression and suicide in adults and children
Z01 AA 00277-02 LCS J. Higley	Non-human primate models of alcohol consumption and aggression
Z01 AA 00278-01 LCS D. George	Behavioral and physiological effects of 2-deoxyglucose infusions
Z01 AA 00279-01 LCS V. Moore	Black and white offspring of parental alcoholics versus control
Z01 AA 00280-01 LCS D. Goldman	Genetic studies of the electroencephalogram and event-related potentials
Z01 AA 00281-01 LCS D. Goldman	Molecular genetic studies on alcoholism in Indians
Z01 AA 00282-01 LCS D. Goldman	Molecular genetic studies on the dopamine D2 receptor
Z01 AA 00283-01 LCS D. Goldman	Molecular genetic studies on enzymes of alcohol metabolism

Z01 AA 00284-01 LCS H.-Y. Kim	Alterations in lipid metabolism in the nervous system by ethanol
Z01 AA 00285-01 LCS J. Karanian	Physiological functions of lipoxygenase products
Z01 AA 00286-01 LCS D. George	Psychobiology of alcoholism in women
Z01 AA 00400-05 USP K. Grant	Selective breeding for ethanol tolerance
Z01 AA 00401-03 LPPS G. Kunos	Interaction between the immune system and adrenergic receptors
Z01 AA 00402-03 LPPS G. Kunos	Brainstem neuromechanisms in blood pressure regulation
Z01 AA 00403-03 LPPS G. Kunos	Inverse regulation of hepatic alpha-1 and beta-adrenergic receptors
Z01 AA 00404-03 LPPS R.L. Kincaid	Control of calcium- and phosphorylation-regulated signaling pathways
Z01 AA 00405-03 LPPS T.M. Martensen	Detection and regulation of specific cellular phosphoproteins
Z01 AA 00406-01 LPPS E. Ishac	Noradrenergic neurotransmission and actions of ethanol
Z01 AA 00479-07 LPPS F. Weight	Synaptic mechanisms and ethanol actions
Z01 AA 00480-07 LPPS F. Weight	Nerve cell excitability and ethanol actions
Z01 AA 00481-01 LPPS L. Takacs	Physiological regulation of IL-1 production
Z01 AA 00482-01 LPPS L. Takacs	Regulation of early steps in T-cell development
Z01 AA 00483-01 LPPS C. Fraser	Molecular biology of G protein coupled receptors
Z01 AA 00484-01 LPPS C. Fraser	Expression of ligand-gated ion channels in xenopus oocytes
Z01 AA 00485-01 LPPS E. Kirkness	Gene structure of GABA-A receptor subunits
Z01 AA 00700-06 LPPS P. Hoffman	Ethanol effects on membrane-bound enzymes

Z01 AA 00702-06 LPPS P. Hoffman	Ethanol modification of neurotransmitter receptor-effector coupling
Z01 AA 00703-06 LPPS P. Hoffman	Neurohypophyseal peptides and ethanol tolerance
Z01 AA 00705-04 LPPS P. Hoffman	In vitro models for ethanol effects on receptor-mediated processes
Z01 AA 00706-02 USP C. Rabe	Effects of ethanol on NMDA-mediated neuronal function
Z01 AA 00707-02 USP K. Grant	Behavioral pharmacology of ethanol

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00024-12 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Metabolic Studies of Human Alcoholics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.L. Veech	Chief	LMMB, NIAAA
Others:	J.P. Casazza	Research Chemist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Department of Academic Medicine, London, England (M. Morgan)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In three separate studies involving three different sets of collaborators, elevated levels of 2,3-butanediol has been found in 80% of the blood samples taken from chronic alcoholics when consuming distilled spirits, but not in social drinkers consuming distilled spirits. 2,3-butanediol has also been identified in two other abnormal metabolic states, patients with congenital propionic and methylmalonic acid uria (Duran M, et al. Clin Chim Acta 1987;165:197-204) and premature infants (Mills GA, Walker V. Clin Chim Acta 1989;179:51-9).

In rat, three experimental models for the production of 2,3-butanediol have been demonstrated. The first involves elevated blood acetaldehyde entering the brain with an active pyruvate dehydrogenase multi-enzyme complex where it condenses with hydroxyethylthiamine pyrophosphate to form acetoin (Veech RL, et al. Curr Top Cell Regul 1981;13:151-79). The acetoin is subsequently converted in liver to 2,3-butanediol. In the second animal model, 2,3-butanediol in the rats is produced by acetone feeding (Casazza JP, et al. J Biol Chem 1984;259:231-6). Prolonged fasting, which results in elevated serum acetone, does not result in 2,3-butanediol production in man. A third model results in the production of 2,3-butanediol after acute administration of short and medium chain fatty acids. The mechanism of production in this case appears to be similar to that observed in acetaldehyde stimulated production of 2,3-butanediol, but in this case pyruvate serves as both the initial substrate and as the acceptor of the hydroxyethyl moiety due to a decreased level of free CoA. Whether 2,3-butanediol production is due to expression of an aberrant gene product or is due to some other metabolic change caused by chronic ethanol consumption is not known, but the presence of D/L diastereomer of this compound during periods of ethanol ingestion in approximately 40% of all alcoholics prior to the onset of alcoholic liver disease and approximately 25% of all alcoholics with alcoholic cirrhosis in the absence of recent ingestion of ethanol

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00036-04 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Regulation of ethanol-inducible cytochrome P450 gene (III)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	B.J. Song	Acting Section chief	LMMB, NIAAA
Others:	R.L. Veech	Chief	LMMB, NIAAA
	Y.P. Yun	Visiting Fellow	LMMB, NIAAA
	J.P. Casazza	Research Chemist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Department of Pediatrics, Albert Einstein Medical School, New York, New York. (Dr. P. Saenger)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

As a continuous ongoing project, the molecular regulatory mechanism of P450IIE was further investigated. We have previously identified and determined the structures of the ethanol-inducible cytochrome P450 (P450IIE1) of both rat and human. We have also demonstrated three distinct types of regulation of P450IIE1 in rat. The three types of up-regulation of P450IIE appeared to be present in liver, lung, and kidney tissues. The mechanism of a negative effect of CCl_4 on P450IIE was further examined. The administration of CCl_4 not only reduced the level of P450IIE associated enzyme activity and the amount of immunoreactive P450IIE, but also caused rapid decline in its mRNA indicating a pretranslational reduction of P450IIE by its own substrate. In contrast, other major classes of P450 did not appear to be affected by CCl_4, indicating a specific reduction of P450IIE. In a collaboratory project with Dr. Casazza, a pretranslational reduction of P450IIE during pregnancy was also observed indicating a negative hormonal control mechanism in P450IIE regulation. The two specific down-regulations of P450IIE by CCl_4 and hormones during pregnancy along with three types of up-regulatory mechanism of P450IIE thus provide an unique example of multiple modes of regulation among the classes of P450, most of which are activated by transcriptional activation.

A method for the measurement of P450IIE1 in easily obtainable human tissues was also established. By immunoblot analysis, P450IIE1 expressed in cultured lymphocytes could be easily detected by specitic antibody to P450IIE1. The levels of P450IIE in lymphocytes from poorly controlled diabetic children are elevated four to ten fold over those of the corresponding control subjects. The induced levels of P450IIE1 determined by the density of immunoreactive bands highly correlated with those of hemoglobin Alc with a correlationship coefficient of 0.87. The levels of P450IIE in lymphocytes from alcoholic patients at admission and discharge were determined by the immunoblot analyses: the levels of P450IIE in alcoholics were four to five folds higher than those found in the voluntary control group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00037-04 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and regulation of genes for pyruvate dehydrogenase multienzyme complex (III)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.	B.J. Song	Section Chief	LMMB, NIAAA
Others:	R.L. Veech	Chief	LMMB, NIAAA
	T.L. Huh	Visiting Associate	LMMB, NIAAA
	J.W. Huh	Visiting Associate	LMMB, NIAAA
	J.P. Casazza	Research Chemist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The data from our laboratory indicated that 2,3-butanediol, one of the unusual metabolites found in human alcoholics, could be generated by the side reaction of pyruvate dehydrogenase (PDH) located in brain and testis. Based on this hypothesis, we have started to study the genes for the PDH multienzyme complex. All the subunits of the PDH complex were purified to near homogeneity from bovine kidney and heart. The purified proteins were subsequently used for antibody generation. We have then cloned and characterized full-length cDNAs for PDH E1a, E1b, and E3 subunits whose genes are located in chromosome X, 3, and 7, respectively. The nucleotide sequence of human brain PDH E1a clone was identical with that of liver clone indicating that the differences in the production of 2,3-butanediol in brain and liver may not be due to a structural difference in PDH E1a but rather due to alteration in metabolic regulators or tissue specific regulation of PDH differently modulated by PDH-specific kinase and phosphatase. Our clones, however, were different from other cDNA clones isolated from foreskin and fetal liver. Two different types of CDNA clones for PDH E1b subunits were also identified and completely characterized. The sequences of our clones were exactly identical, but they are different from that isolated from foreskin in several regions. The correct sequences of our clones were verified by a method of polymerase chain reaction and DNA sequencing. The differential regulation of PDH complex was also studied in cultured human fibroblasts from patients of lactic acidosis. In two cell lines of Leigh syndrome patients, no abnormality of PDH complex was observed while a major problem in PDH complex was observed in a TCA-cycle defective cell line. The defect in these cells was determined to be related to problems of PDH protein translation and processing. PDH-specific protein kinase and phosphatase were also purified and their N-terminal amino acid sequences were determined. Based on the partial amino acid sequences, several oligodeoxynucleotides were synthesized and used to clone the genes coding for PDH-specific kinase as well as PDH-phosphatase, whose structures and regulation were not characterized yet.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00038-03 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NMR Studies of Cerebral Blood Flow and Energy Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	A.C. McLaughlin	Section Chief	LMMB, NIAAA
Others:	L. Ligeti	Visiting Scientist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

BEIP, NIH (C. Moonen); NINDS, NIH (J. Alger); NHLBI, NIH, Georgetown University (P. Van Zijl); Children's Hospital of Philadelphia (L. Sutton)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.40	0.40	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was undertaken to assess a new method for the non-invasive determination of cerebral blood flow without the use of radioactive tracers. Specifically, we used a new 19F NMR technique to measure the clearance of a fluorinated inert gas, CHF3, from the brain. We tested the new technique by comparing the cerebral blood flow values determined by 19F NMR with cerebral blood flow values determined simultaneously by radioactive microsphere techniques. The cerebral blood flow values determined by 19F NMR and radioactive microsphere techniques agreed reasonably well, and showed the same response to variations in the arterial CO2 level. We conclude that the 19F NMR technique gives a quantitative measure of cerebral blood flow.

In the last year we used NMR imaging techniques to localize the 19F NMR spectra (and thus the blood flow measurement) to a well defined region of the brain. We also began a series of experiments that combines 19F NMR determination of the cerebral blood flow (and oxygen consumption) with 31P NMR determination of cerebral energetics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 0039-03 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cerebral Blood Flow and Energy Metabolism in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	A. C. McLaughlin	Section Chief	LMMB, NIAAA
Others:	E. Dora	Visiting Scientist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.20	1.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of humoral and neuronal factors in the control of cerebral blood flow and metabolism is controversial. We have studied the role of the adrenal/hypophysis axis and the role of sympathetic activation on cerebral blood flow and metabolism under normal and stressful conditions. Our initial work has been done with the hypercapnic model for stress, but the studies will be extended to other models for stress, e.g., hypoxia, hypoglycemia and withdrawal from chronic alcohol consumption.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00040-03 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Electrostatic Properties of Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	A.C. McLaughlin	Section Chief	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Physiology Department, State University of New York, Stonybrook, NY (S. McLaughlin); Biochemistry Department, University of Pennsylvania, Philadelphia, PA (J.R. Williamson).

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.9	0.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The surface potential of cellular membranes is an important determinant in the physiological function of the cell. We have investigated a number of factors that affect the surface potential of membranes. We have also modified the theory that has been used to calculate the surface potential, the Gouy-Chapman theory, to account for these factors.

Triphosphoinositide lipids in plasma membranes could have up to five negative charges. We previously investigated the interaction of calcium, magnesium, potassium, protons and other cations with triphosphoinositides, and determined the number of cations bound to the lipid under physiologically-relevant conditions. We also investigated proton titration curves of the lipid and found that there was a cooperative interaction between the two monoester groups in the inositol headgroup. We proposed that this interaction could have important consequences in terms of the net charge on the triphosphoinositide lipid under physiological conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00041-03 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determination of Plasma Free Magnesium Concentration by Ion-Selective Electrodes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: A. C. McLaughlin Section Chief LMMB, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.50	0.20	0.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An ion-selective technique has been developed for the determination of free serum magnesium levels. A number of major technical difficulties have been overcome, but further studies to determine the accuracy of the technique are necessary.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00042-02 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Multiple Quantum NMR Studies of Sodium and Potassium in the Rat Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	A. C. McLaughlin	Section Chief	LMMB, NIAAA
Others:	R. Lyon	Staff Fellow	LMMB, NIAAA
	L. Ligeti	Visiting Scientist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

BEIP, NCRR, NIH, Bethesda, MD (J Pekar, CT Moonen)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.20	1.20	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously developed a new approach for studying trans-membrane ion gradients in the intact brain utilizing the different NMR relaxation times of intracellular and extracellular ions. Double quantum NMR spectra are more sensitive than conventional single quantum NMR spectra to changes in relaxation times. Double quantum and single quantum 23Na and 39K NMR spectra were obtained from rat brain in vivo. Upon death, the double quantum 23Na NMR signal increased by a factor of three, while the single quantum signal decreased by 20%. The results are consistent with the well-known influx of sodium ions into the cell, and suggest that double quantum sodium and potassium NMR may be useful in visualizing compromised regions of the brain.

In the last year we have extended this approach in a number of directions. First we extended the approach to study the cat brain in vivo, the rat leg in vivo, and the perfused rat liver. Second, we used double-quantum 23Na NMR to study compartmentation of sodium in the intact brain. Third, we combined multiple-quantum 23Na NMR with 31P NMR to study the correlation between changes in the multiple-quantum sodium NMR signals and changes in cellular energetics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00043-02 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effects of Ethanol on Isolated Cerebral Arteries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E. Dora	Visiting Scientist	LMMB, NIAAA
Others:	A. C. McLaughlin	Section Chief	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Experimental Research Department, Second Institute of Physiology, Semmelweis Medical University, Budapest, Hungary (Farago, M)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.20	0.20	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The acute administration of ethanol decreases cerebral blood flow in the intact animal (see accompanying Project Report "Cerebral Blood Flow and Metabolism in the Rat"). The in vivo effects of ethanol can bee studied in more detail with the isolated artery preparation. Studies with isolated arteries have shown:

(1) Ethanol induces contractions in isolated arteries, and facilitates the vasoconstrictory effects of norepinephrine.

(2) The effects of ethanol appear to be specific for cerebral arteries.

(3) Preliminary results suggest that ethanol may interfere with the endothelium-mediated regulation of cerebral vascular smooth muscle tone.

This project has been terminated.

Publications:

Dora E, Feher E, Farago M, Horvth I, Szabo C. Mechanism of hemoglobin-induced spasm in the isolated middle cerebral artery of the cat, Adv Exp Med Biol 1989;248:533-42.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00044-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Site Directed Mutagenesis to Study the Mechanism of Action of Pig Citrate Synthase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.P. Casazza	Research Chemist	LMMB, NIAAA
Others:	None		

COOPERATING UNITS *(if any)*

Basic Biochemistry Department, VA Medical Center, Dallas (P. Srere, C. Evans) and Department of Biological Chemistry, Wayne State University School of Medicine (G. Alter).

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Prior to the development of molecular biology physicochemical studies of enzymes were limited to studying native enzymes, proteolysed enzymes and in certain cases chemically modified enzymes. With the advent of site directed mutagenesis and bacterial expression vectors with strong promoters, it has become possible to ask questions about the role of specific amino acids in catalysis, selectively change these amino acids, produce large amounts of the mutant protein in culture, and then study the physicochemical properties of the purified protein. We have modified His274 and Asp375 in pig citrate synthase, two amino acids identified as being at the active site of the enzyme by X-ray crystallography. These amino acids were replaced not only with uncharged amino acids, but also with amino acids with different pKa's, charge and chain lengths. Kinetic analysis of these mutant enzymes along with already published crystallographic data suggest that, (1) prior to the actual condensation reaction His274 and Asp375 act in concert, maintaining the electrostatic neutrality of the His-Asp pair while facilitating the enolization of acetyl CoA without the formation of the enolate ion and (2) during the actual condensation reaction His274 accepts a proton from the hydroxide of the enol intermediate as ASP375 protonates the reaction His274 accepts a proton from the hydroxide of the enol intermediate as Asp375 protonates the thioester sulfer, forming the sultonium ion, thus facilitating the displacement of coenzyme A by oxaloacetate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00045-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vivo Portal-Hepatic Venous Gradients of Glycogenic Precursors in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G. P. Dobson	Visiting Associate	LMMB, NIAAA
Others:	R. L. Veech	Chief	LMMB, NIAAA
	J. V. Passonneau	Research Chemist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Department of Biochemistry, Chang Gung Medical College, Tao-Yuan, Taiwan R.O.C. (M.T. Huang)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.6	.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Following the pioneering experiments in the mid-nineteenth century of Claude Bernard, it was fairly well established that dietary glucose in the post-prandial state was taken up directly by the liver and incorporated into newly synthesized glycogen. About 10 years ago this viewpoint was strongly contested by the radiotracer studies of Newgard et al., (JBC 1984;258:8046-52), later to be supported by Drs. J. Katz and B. Landau. Their position was that the metabolic fate of dietary glucose was not the direct incorporation into liver glycogen, but rather it was first broken down to C3 units by unspecified organs of the body which then returned to the liver to form glycogen via gluconeogenesis. This latter pathway was called the "Indirect Pathway", and an entirely new field of inquiry termed "The Glucose Paradox" was born. We challenged this interpretation and carried out experiments on chronically cannulated male Wistar rats which were fed ground chow for 2 hr for six days. On the 7th post-operative day, blood was simultaneously drawn from the portal and hepatic veins over a 2 hr feeding period. The position of the hepatic vein cannula was verified using a tritiated water washout technique. In separate experiments, 200 uCi of [3-^{3}H]glucose was added to the food in order to determine the relative contribution of D-glucose and 3-C precursors to newly synthesized glycogen. Our results demonstrate that 73% of newly synthesized liver glycogen formed during the 2 hr of feeding came directly from the uptake and phosphorylation of dietary glucose from the gut via the portal vein without prior conversion to 3-C precursors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00046-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quanitation of 31P NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G.P. Dobson	Visiting Associate	LMMB, NIAAA
Others:	R.L. Veech	Chief	LMMB, NIAAA
	J.V. Passonneau	Research Chemist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Dept. Biochem. Biophys., University of Pennsylvania, PA (K Kobayashi, T Inubushi, S Wehrli, S Nioka, B Chance)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Despite 31P NMR being a potentially important tool to non-invasively monitor tissue metabolism in states of health and disease, no single study has addressed the quantitative aspects of the method by comparing it to conventional chemical methods. Hearts from 200-350 g fed male Wistar rats were perfused at low and high workloads with Pi-free Krebs-Henseleit medium containing either 10 mM glucose or 10 mM glucose plus 15 mU/ml insulin. The intracellular pH by 31P NMR ranged between 6.99 and 7.02 and agreed to within 0.1 pH unit of estimates calculated using enzymatically determined total tissue HCO3-/CO2 contents. At high work, where the tissue contents of PCr and ATP were determined on the same heart as NMR areas (n=16), the proportionality factors, defined as the 31P NMR area units divided by the total enzymatic tissue content (area units/umol g-1 wet wt heart), was 112 $\pm$ 8 for PCr; 99 $\pm$ 4 for g-ATP; 138 $\pm$ 9 for a-ATP and 100 $\pm$ 4 for b-ATP. It was concluded that ATPO and PCr were equally visible by 31P NMR and that no significant breakdown of PCr from tissue acid extraction occurred. The other part of the study focused on the estimation of cytosolic Pi. The cytosolic Pi estimated from NMR in glucose-perfused hearts at low and high work was 0.92 $\pm$ 0.11 and 0.92 $\pm$ .08 umol/g wet wt. Using the near-equilibrium expression of KCK/KG+G and the metabolite levels in heart extracts, the calculated cytosolic Pi was 1.45 $\pm$ 0.19 and 3.26 $\pm$ 0.50 umol/g wet wt respectively. The cytosolic NMR Pi in the glucose plus insulin hearts was 0.54 $\pm$ 0.07 and 0.76 $\pm$ 0.05 umol/g wet wt at low and high work and 1.57 $\pm$ 0.22 and 1.94 $\pm$ 0.37 umol/g wet wt from near-equilibrium estimates. The total tissue Pi measured enzymatically ranged from 2.16 $\pm$ 3.17 umol/g wet wt heart. The validity of using both the NMR and near-equilibrium method for estimating cytosolic Pi in the heart was discussed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00047-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of alcohol and acetate on leukocyte membrane receptor expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.M. Jones	Visiting Scientist	LMMB, NIAAA
Others:	R.L. Veech	Chief	LMMB, NIAAA
	B.J. Song	Senior Staff Fellow	LMMB, NIAAA
	Y.P. Yun	Visiting Fellow	LMMB, NIAAA

COOPERATING UNITS *(if any)*

Francis Scott Key Medical Center, Johns Hopkins University, Baltimore, MD (G. Briefel).

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [x] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose was to determine whether chronic exposure to moderate to high blood levels of acetate would result in alterations of expression of selected membrane markers. For this purpose, we examined alcoholic subjects exposed to acetate via alcohol metabolism (up to 10 fold physiological levels) and hemodialysis patients exposed via the dialysis buffers (up to 100 fold physiological levels). Acute exposure to high levels of alcohol was reported by others to enhance the expression of HLA class I antigens. In the present study, the expression of HLA class I (ABC), Class II (Dr) and CD16 Fc receptor antigens were measured by flow cytometry on blood leukocytes from alcoholic and hemodialyzed uremic patients. By staining with fluor=labeled monoclonal antibodies, we detected a small but not significant increase in expression (intensity) of HLA class I antigens on lymphocytes and monocytes of alcoholic subjects in comparison with a reference population of normal controls. Hemodialysis subjects exhibited a decreased expression of HLA class I. Alcoholic and hemodialysis subjects both showed a significant decrease in expression of HLA class II antigens. The average percentage of lymphocytes from alcoholics that expressed CD16 was similar to that of controls. Hemodialyzed subjects exhibited a decrease percent of lymphocytes that stained for CD16 but an increase percent of monocytes that stained for CD16. The results were compatible with a hypothesis that altered expression of HLA class I reported by others was an alcohol effect while the altered expression of class II and CD16 that we observed was an acetate effect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00048-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Distribution in the Perfused Rat Hearts: Effect of Pi and Ethanol

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	T. Masuda	Visiting Fellow	LMMB, NIAAA
Others:	G.P. Dobson	Visiting Associate	LMMB, NIAAA
	R.L. Veech	Chief	LMMB, NIAAA
	K. Sato	Visiting Fellow	LMMB, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Isolated hearts were perfused in modified Krebs-Henseleit buffer (118 mM NaCl, 5.9 mM KCl, 1.07 mM free Ca2+, 0.5 mM free Mg2+, 25 mM NaHCO3, and 10 mM glucose, pH 7.4 at 38oC) containing 0, 1.2 or 5 mM Pi. The total H20 content was 0.853 ml/g wet wt. of which 59.3% (14C-mannitol space) was extracellular and independent of [Pi]. No significant change in intracellular Pi content was found with values ranging from 15.8 to 20.5 umol/ml cell H20. Increasing Pi from 0 to 5 mM significantly decreased total [Mg2+] from 17.0 to 16.2 umol/ml. From the citrate/isocitrate ratio, free intracellular [Mg2+] was 0.36, 0.44 and 0.04 umol/ml for 0, 1.2 and 5 mM Pi. The calculated intracellular pH from tissue HCO3- was 6.98, 7.19 and 7.15 respectively. As Pi was increased from 0 to 5 mM, the [Cl-] decreased from 51.7 to 11.6 umol/ml. In contrast, intracellular [K+] was invariant at 128 to 132 umol/ml giving a membrane potential of about -83 mV. The intracellular [Na+] with increasing Pi was 1.62, 3.68 and 2.03 umol/ml. The cytosolic [ATP]/[ADP][Pi] from 3PGK-GAPDH-LDH equilibria decreased from 9,300 M-1 to 3,360 M-1 in the 0 mM and 5 mM Pi perfused hearts. The free energy of ATP hydrolysis was found to be -13.1, -13.3 and -13.2 kcal/mole for 0, 1.2 and 5 mM Pi hearts. The energy of the plasma membrane gradients of [Na+]o3[K+]i2[Cl-]o/[Na+]i3[K+]o2[Cl-]i were +12.7, +11.3 and +13.2 kcal/mole, or 97%, 85% and 99% of the cellular phosphorylation potential. It was concluded that the extent and direction of the major inorganic ions and membrane potential across constitute a Gibbs-Donnan equilibrium system catalyzed by transport enzymes sharing common substrates. The chemical and electrical energies of those gradients are equal in magnitude and opposite in sign to the chemical energy of ATP hydrolysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00049-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymes and Metabolites of the Purine Pathway in Rat Liver

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R.L. Veech	Chief	LMMB, NIAAA
Others:	M.T. King	Research Chemist	LMMB, NIAAA
	J.V. Passonneau	Research Chemist	LMMB, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Metabolic Control

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.6	1.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purine pathway in liver is being investigated with relation to the effects of alcohol. The administration of alcohol is known to result in elevated uric acid levels, uric acid turnover, and hyperuricemia. Subsequent to alcohol ingestion or administration, there is an increase in acetate, changes in the blood phosphate levels, and phosphate and pyrophosphate concentrations in liver tissue. We are investigating, in a systematic fashion, the effects of ethanol plus or minus an inhibitor of ethanol metabolism (4-methyl-pyrazole), and the products of ethanol metabolism acetate and phosphate. The substances are administered to rats, and the livers quick frozen after 30 min. Investigations have been initiated of the effects of these compounds on the metabolites which are involved in purine biosynthesis; the nucleobases and nucleotides; and the key enzymes in the synthetic pathway. Preliminary results indicate that alcohol administration results in the elevation of pentose pathway metabolites, and phosphoribosyl pyrophosphate, which is the rate-limiting substrate in the first step in purine biosynthesis. New methods have been developed for the measurement of metabolites, nucleobases and enzyme activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00050-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Relationship of Cytokines to Alcoholic Liver Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: R.L. Veech Chief LMMB, NIAAA

COOPERATING UNITS *(if any)*

Dept. of Medicine, John Hopkins University, Baltimore, MD (E. Mezey); Cleveland Clinic Foundation, Cleveland, Ohio (M. Felver)

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.1	.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three cytokines, tumor necrosis factor (TNFα) and interleukin 1α and β (IL-1α and β), were studied for their relationship to alcoholic hepatitis. In a retrospective study of 23 patients with alcoholic hepatitis we found that elevated plasma levels of TNFα either at admission or discharge from the hospital were associated with death in 82% (14/17) of patients. In contrast, absence of elevated plasma TNFα was associated with survival in 100% (6/6). Plasma TNFα levels were also studied in alcoholic patients without clinically apparent liver disease, with alcoholic cirrhosis, or in nonalcoholic healthy controls and no elevated levels were found. Plasma IL-1α levels were also significantly increased in alcoholic hepatitis patients on admission and discharge whereas plasma IL-1β levels were not. Neither IL-1α nor β was correlated with outcome in the alcoholic hepatitis group. We have concluded that the presence of elevated plasma TNFα is a significant predictor of decreased long-term survival in patients with severe alcoholic hepatitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00051-01 LMMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Effect of Ethanol on Cerebral Blood Flow and Energy Metabolism in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	A. C. McLaughlin	Section Chief	LMMB, NIAAA
Others:	L. Ligeti	Visiting Scientist	LMMB, NIAAA
	Z. Ruttner	Visiting Fellow	LMMB, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Metabolism and Molecular Biology

SECTION

Physical Chemistry

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We developed a new technique for measuring cortical cerebral blood flow in the rat, and combined it with measurements of arterial/venous differences for oxygen and glucose to calculate cerebral oxygen consumption and cerebral glucose consumption. The major advantage of this new technique is that it can be used in the unanesthetized, freely-moving, animal. Most of the previous studies on the effect of alcohol on cerebral blood flow and metabolic rate have been performed on restrained or anesthetized animals, where it is difficult to separate the effects of stress and anesthesia from the effects of alcohol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00231-08 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Central and Peripheral Nervous System Function in Abstinent Alcoholics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Eckardt	Section Chief	LCS, NIAAA
Others:	J. Rohrbaugh	Research Psychologist	LCS, NIAAA
	T. George	Section Chief	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA
	D. Flowers	Special Volunteer	LCS, NIAAA

COOPERATING UNITS *(if any)*

George Washington University (H. Weingartner); Biol. Psychiat. Br., NIMH (P. Gold).

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Science

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	2.5	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Behavioral deficits in alcoholics have been conceptualized in terms of two neuropathologically distinct syndromes: alcoholic dementia and Korsakoff's psychosis (alcohol amnestic disorder). Alcoholic dementia is characterized by diffuse cortical damage primarily related to the neurotoxicity of alcohol; Korsakoff's psychosis is associated with subcortical lesions due to nutritional (thiamine) deficiency. Severe memory impairment with relative sparing of other intellectual functions distinguishes Korsakoff's psychosis from alcoholic dementia (which may be clinically indistinguishable from the most common cause of dementia, Alzheimer's disease). We have recently found that sleep in Korsakoff patients is characterized by a reduced REM latency compared to normal volunteers, whereas Alzheimer patients have normal latencies. Furthermore, delta sleeps reduced in Alzheimer's disease, but is normal in Korsakoff's psychosis. Most patients with demonstrated reduced daily excretion of the major urinary metabolite of melatonin, hydroxmelatonin, in patients with Korsakoff's psychosis. This finding is suggestive of impaired pineal function, Genetic differences in thiamine metabolism may predispose patients to develop Korsakoff's psychosis. Most patients with Korsakoff's psychosis whom we have studies have had a transketolase with reduced affinity for thiamine pyrophosphate. The majority of alcoholics with cognitive modulation of neurotransmitter systems may be effective in treatment strategies subcortical syndrome, whereas alcoholic dementia may require treatment strategies similar to those in Alzheimer's disease. This protocol is intended to utilize clinical, neuroradiological, physiological, and neuropharmacological tests to differentiate these two pathologic entities, to follow a longitudinal course, and to relate variables in treatment protocols to outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00233-08 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Family Studies of Alcoholism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Brown	Unit Chief	LSC, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	D. George	Acting Section Chief	LCS, NIAAA
	V. Moore	Social Worker	LCS, NIAAA
	D. Goldman	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Social Work Department, Clinical Center, NIH (E. Hofstetter); NIMH (D. Garnett)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Science, Unit of Family Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Unit of Family Studies has two major functions (1) to recruit and assess alcoholics, controls and their families, for various investigators within the Laboratory of Clinical Studies; and (2) to conduct psychosocial and psychobiological studies of alcoholic families and their individual members. The Unit staff continues to focus on coding and entering onto a computer the data collected since the inception of the Laboratory. A series of correlational studies comparing suicidal versus non-suicidal alcoholics on clinical, psychosocial and family variables has been carried out. In addition, the Unit has collected data on the adult offspring of alcoholics and non-alcoholics, black and white, respectively; pilot efforts for a follow-up of matched black and white former inpatients of our inpatient unit has begun. Unit staff has also been collaborating with the Unit on Genetic Studies in identifying and phenotyping several pedigrees for linkage analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00234-08 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Studies of Serotonin Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Goldman	Section Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	R. Haber	NRC Fellow	LCS, NIAAA
	W. Chen	Senior Staff Fellow	LCS, NIAAA
	B. Giblin	NRC Fellow	LCS, NIAAA
	J. Stoll	Senior Staff Fellow	LCS, NIAAA
	R. Lister	Visiting Scientist	LCS, NIAAA
	G. Brown	Unit Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

NIAID, NIH (C. Kozak); Clemson University (E. Pivorun), Program Resources Inc., Frederick, MD (M. Dean); University of Helsinki (M. Virkkunen); VA Medical Center Portland, OR (J. Crabbe)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section on Genetic Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.5	4.5	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To identify genetic loci determining alcoholism, we have focused on human and murine behaviors associated with serotonin function. We have studied candidate genetic loci involved in serotonin function including tryptophan hydroxylase, serotonin receptors and the serotonin transporter. After cloning tryptophan hydroxylase from mouse mastocytoma cells, we showed that this cDNA recognized brainstem mRNA for this rate-limiting enzyme of serotonin synthesis. The TrpOHas gene was located to mouse chromosome 7. Cloning and sequencing of the 21 kilobase gene allowed the delineation of its putative regulatory sequences as well as its intron/exon boundaries. Genetic studies of the serotonin receptors have been performed in deer mice. The serotonin transporter was functionally expressed in Xenopus oocytes, allowing us to approach the molecular cloning of this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00235-09 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nutritional Effects on Lipid Composition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Salem	Section Chief	LCS, NIAAA
Others:	H.-Y. Kim	Senior Staff Fellow	LCS, NIAAA
	M.J. Bossant	Visiting Fellow	LCS, NIAAA

COOPERATING UNITS *(if any)*

Dept. of Clinical Pathology, Vanderbilt Univ. (H. Knapp)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Analytical Chemistry

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is our hypothesis that alcohol abuse leads to the depletion of polyunsaturated lipids from many tissues and leads to altered metabolism of eicosanoids. Such changes in the liver as well as in other organs and tissues may cause or contribute to the pathological changes associated with alcoholism. Losses in arachidonic acid (20:4n6) and other n-6 fatty acids have been observed in our studies of alcohol exposed rat tissues as well as in the blood cells of alcoholics. This led to the question of whether it was possible to replace these essential fatty acids by dietary manipulation.

It was first established that the fatty acid profiles of various tissues could be drastically altered by manipulation of the type of dietary fat. Rats were fed corn, olive, borage or fish oil based diets for 10-28 days and the lipid profiles with respect to the n-3/n-6 ratio could be changed 10-fold or more in peripheral tissues. In the brain, dietary challenge produced a much less marked effect but this ratio was still significantly altered. The fish oil diet was shown to lead to the production of PGI3 metabolites in the urine for the first time in rats while not affecting PGI2 production. Dietary supplementation with the n-6 precursor gamma-linolenic acid, 18:3n6, could, in some cases, prevent the loss in 20:4n6 caused by ethanol and this may have a possible therapeutic value for alcoholics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00237-08 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Individual Variability in Drug Metabolism by Carbon Dioxide Breath Test

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Shoaf	Senior Staff Fellow	LCS, NIAAA
Other:	T. Hart	Chemist	LCS, NIAAA

COOPERATING UNITS *(if any)*

Epilepsy Branch, NINDS (R. Porter); Nursing Department, CC (I. Naveau)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The caffeine-carbon dioxide breath test may be a useful nonivasive measure of oxidative drug metabolism and liver function in alcoholic patients. 13-C-labeled methyl groups in caffeine are removed and further oxidized to 13-CO-2, which was measured in expired breath samples by isotope ratio mass spectrometry. Single 100mg doses of caffeine were administered to 10 alcoholic patients, at intervals from 2 to 73 days after the patients' last alcoholic drink. The breath test results were expressed as the cumulative amount of 13-CO-2 expired in 2 hrs. The results of tests in 6 smokers and 4 nonsmokers were compared and the changes in results in each patient were examined. The results of the first test (2 to 7 days after the last drink) were, on the average, 3.8 times higher in smokers than in nonsmokers($p \leq 0.01$),indicating more rapid metabolism in smokers. Over time, the breath test results increased more than 20% in 2 smokers and 3 nonsmokers, but decreased more the 20% in test result was in-creased to >200% of the first measurement and in 2 patients to 150% - 200%. The maximum decrease observed was to 57% of the first measurement. It appears that this test can be used to detect clinical alcoholics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00238-08 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CSF Neuropeptides and Prostaglandins in Alcohol Withdrawal and Brain Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. Linnoila Chief LCS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Clinical Neurogenetics, NIMH (W. Berrettini); VA Medical Center, Washington, D.C. (J. Hawley), Department of Psychiatry, Duke University Medical Center (C. Neueroff; G. Bissette).

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Severity of withdrawal symptoms from alcohol was quantified in alcoholics admitted to the Neurology Ward of the Washington, D.C. VA Hospital. Cerebrospinal fluid (CSF) samples were repeatedly obtained early during withdrawal and after all symptoms had subsided. Concentrations of the monoamine neurotransmitter norepinephrine and its major metabolite MHPG were measured at NIH. Significant positive correlations were observed between indices of elevated norepinephrine turnover and several signs of alcohol withdrawal. We are continuing this work trying to identify causes for the noradrenergic dysregulation during alcohol withdrawal. Thus, we are measuring peptides known to synapses simultaneously with norepinephrine. We are correlating norepinephrine and MHPG in the CSF and to the severity of withdrawal symptoms in our patients. We are continuing to increase our sample size. Our new mass spectrometric method to quantify glandius has shown that the concentrations of prostaglaudins is human lumbar CSF are negligible. Thus, previous quantification by radioimmuno assays are probably erroneous.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00239-07 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alcoholism-Associated Cognitive Impairment and Organic Brain Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Eckardt	Section Chief	LCS, NIAAA
Others:	E. Joyce	Visiting Associate	LCS, NIAAA
	R. Lister	Visiting Scientist	LCS, NIAAA
	R. Rawlings	Mathematical Statistician	DBE, NIAAA

COOPERATING UNITS (if any)

United States Soldiers' and Airmen's Home, Washington, D.C. (A. Law);
George Washington University (H. Weingartner)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Brain Research

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN YEARS	PROFESSIONAL	OTHER
1.5	.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to examine the neuropsychological performance of several clinically defined populations of detoxified male alcoholics. Comparisons will be made among detoxified alcoholics with clinically defined chronic organic brain syndromes, demetia or amnestic syndrome less cognitively impaired alcoholics who are in alcoholism treatment programs; and nonalcoholic controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00240-11 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cognitive Function in Male Alcoholics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Eckardt	Section Chief	LCS, NIAAA
Others:	R. Rawlings	Mathematical Statistician	DBE, NIAAA

COOPERATING UNITS *(if any)*

Department of Psychiatry and Human Behavior, Univ. of California, Irvine (L. Gattschalk)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Brain Research

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This series of studies is concerned with cognitive function in detoxified male alcoholics. Recent and chronic alcohol consumption variables were found to interact with each other and with age and education in a non-liner fashion in predicting neuropsychological performance. Increased consumption predicted decreased performance, even on tests whose mean scones were in the normal range. Little or no improvement in performance was demonstrable with short-term abstinence (14-20 days), although long-term abstinence (7 months) was associated with improvement. Similarly, hepatic and hematologic characteristics of long-term abstainers improved, whereas these systems functioned abnormally in people who continued to consume alcoholic beverages, albeit at significantly reduced levels. Relationships between various pretreatment prediction variables and subsequent outcome are also being studied. Increased risk of relapse was associated with excessive drinkers who were relatively early in their alcoholic careers as assessed by years of abusive drinking and accumulated lifetime exposure to alcohol. Although statistically significant relationships were observed between scores on certain neuropsychological tests and posttreatment alcohol consumption, neuropsychological evaluation was determined to be of limited clinical utility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00249-07 LCS

PERIOD COVERED

Octorber 1. 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacologic Reduction of Alcohol Consumption in Alcoholic Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Section Chief	LCS, NIAAA
Others:	M. Eckardt	Section Chief	LCS, NIAAA
	R. Eskay	Research Physiologist	LCS, NIAAA
	M. Linniola	Chief	LCS, NIAAA
	N. Salem	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Stuides

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies indicate that alcohol consumption is regulated 2by several interacting neurotransmitters, including the dopamine and serotonin systems. In a randomized double-blind design, chronic alcoholic outpatients received L-DOPA or L-5-hydroxytrytophan, both with the peripheral decarboxylase inhibitor carbidopa or placebo for a one year period. During this year, alcohol consumption, liver function, craving for alcohol, mental status, psychosocial functioning, and compliance with medication were assessed at regular intervals. Prior to entry into the study, after 3 months, and at one year, the following procedures were conducted to measure drug effects: (1) behavioral evaluation; (2) determination of concentrations of drugs, monoamines, hormones, and peptides in blood and cerebrospinal fluid; (3) orthostatic changes in heart rate, blood pressure, and plasma norepinephrine concentrations; and (4) assessment of plasma vasopressin response to saline infusion. Changes in alcohol consumption will be related to biochemical and behavioral parameters.

This study has been completed. The results are being analyzed and will be submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00250-07 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrophysiological Studies of Acute and Chronic Alcohol Consumption

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	J. Rohrbaugh	Research Psychologist	LCS, NIAAA
Others:	M. Eckardt	Section Chief	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA
	D. Rio	Research Physicist	LCS, NIAAA
	R. Momenan	Visiting Fellow	LCS, NIAAA
	M. Enoch	Visiting Fellow	LCS, NIAAA

COOPERATING UNITS *(if any)*

Department of Psychology, Catholic University (R. Parasuraman); Department of Electrical Engineering, University of Nebraska (J. Varner)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Brain Research

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.2	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this research is to study the covert brain processes that underlie cognition and performance in human subjects, and the acute and chronic effects of ethanol upon such processes. Included is an extensive study in which we are examining brain processes in individuals with different family histories of alcoholism. A principal focus of these studies is the measurement of brain electrical potentials, which provide information regarding the timing and character of the constituent sensory, cognitive and motor elements that are the mechanisms underlying observable behavior. The study of the brain potentials also allows inference of the specific brain regions affected by ethanol. The brain electrical potentials are studied within a broad context provided by performance and psychometric data, and measurement within other psychophysiological response systems.

We have obtained data which document a large number of acute and chronic effects on specific brain functions, ranging from sensory input to motor control functions. Of particular interest is a finding that brain electrical and autonomic signs of alerting and orienting are enhanced by ethanol, in contrast to its depressant effect on most other functions. A similar effect was observed in a sample of chronic alcoholics organic brain disorder patients. Such findings suggest that ethanol intoxication and alcoholic organic brain disease may be associated with a disinhibition or deregulation of orienting processes. The attendant fragmentation associated with alcohol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00257-06 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Neuroendocrine Studies in Offspring of Familial Alcoholics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	G. Brown	Unit Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Responses of thyroid stimulating hormone (TSH) to thyrotropin releasing hormone (TRH) have been studied in the offspring of familial alcoholics and age, sex, and past alcohol exposure matched control children. Sons but not daughters of familial alcoholics were found to have exacerbated TSH responses to TRH infusions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00258-06 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Violent Behavior, Neruotransmitters, Glucose Metabolism and Alcohol Abuse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	M. Linnoila	Chief	LCS, NIAAA
Other:	D. Goldman	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Department of Psychiatry, University Central Hospital, Helsinki, Finland (M. Virkkunen); Office of the Administrator (F. Goodwin)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

We have investigated neurotransmitter metabolites and glucose metabolism in incarcerated violent offenders, arsonists and healthy volunteers. We have found that low cerebrospinal fluid (CSF), 5-hydroxyindoleacetic acid (5HIAA) concentrations and hypoglycemia during oral glucose tolerance tests are associated with each other and impulsive violent acts and fire setting. In a follow up study we found that a low blood glucose nadir and low CSF 5HIAA concentration were powerful predictors of recidivism among impulsive violent offenders and fire setters. We are currently collecting lymphocytes for molecular genetic studies from violent offenders, their family members and appropriate controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00262-07 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Fatty Acid Metabolites by GC/MS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Salem	Section Chief	LCS, NIAAA
Others:	R. Pawlosky	Senior Staff Fellow	LCS, NIAAA
	H-Y Kim	Senior Staff Fellow	LCS, NIAAA
	S. Sawazaki	Visiting Fellow	LCS, NIAAA

COOPERATING UNITS *(if any)*

Dept. of Clinical Pathology, Vanderbilt Univ, (H. Knapp)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Analytical Chemistry

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Desaturation is a necessary step in the modification of dietary linoleic and linolenic acids to arachidonic and docosahexaenoic acid, respectively. The levels of these acids are lowered by ethanol in the liver and brain and it is likely that this leads to some of the pathological consequences associated with alcoholism. We are therefore examining the basic biochemistry of the desaturase system and observing the effects of both diet and alcohol exposure on enzymatic activity. The initial phase of this work requires the development of methodology for assay of desaturase activity both in vivo and in vitro using stable isotopically labeled fatty acids by selected ion monitoring electron capture negative ionization GC/MS. The characterization of the chromatographic, electron impact , and negative ion electron capture mass spectra of the pentafluorobenzyl (PFB) derivatives of the various fatty acids has been initiated. Methods for producing the PFB derivatives from polar and neutral lipids as well as subsequent purification procedures and recovery experiments are under development. In vitro and in vivo studies with rats, mice,and guinea pigs will provide a basis for the development of human protocols. We investigated the effects of both lipid dietary modifications and chronic ethanol exposure on the production and release of 2-series prostaglandins from rat brain slices. Rats were given diets with different fat sources for two weeks and exposed to alcohol via inhalation for an additional week. Results of the in vitro procedure showed that both diet and ethanol treatments significantly affected prostaglandin release from brain slices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AA 00265-05 LCS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Alprazolam, Diazepam, Clonidine, and Placebo upon Ethanol Withdrawal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	K. Immerman	Surgeon	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Clinical Studies

SECTION
Section of Clinical Science

INSTITUTE AND LOCATION
NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ethanol withdrawal syndrome, ehich is characterized by an increased activity of the noradrenergic system, is at present most commonly treated with diazepam or chordiazepoxide, both conventional bezodiazepines. Alprazolam, a triazolobenzo-diazepine, has been demonstrated to be efficacious in the pharmacotherapy of depression and anxiety disorders, in contrast to the conventional lbenzidiazepines. Alprazolam may have a particularly potent inhibitory action on the

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00266-05 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relationship of Psychobiology to Psychopathology in Alcoholics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Section Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	M. Eckardt	Section Chief	LCS, NIAAA
	D. Goldman	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Clinical Psychobiology, NIMH (W. Potter); Biological Psychiatry, NIHM (T. Uhde)

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Laboratory of Clinical Studies

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Section of Clinical Science

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alcoholism and affective disorders frequently occur in the same individuals and in members of the same family. This association may represent the co-existence of two common disease entities due to chance or due to (a) alcoholism resulting from self-medication of an underlying affective disorder, or (b) depression resulting from toxic effects of alcohol abuse. Studies have shown that alcohol may acutely improve the sense of affective well-being, but with continued intoxication this improvement may be reversed. Also, during chronic experimental intoxication, alcoholics not only become increasingly depressed but also more anxious.

In this protocol we propose to characterize certain biochemical aspects of depression and anxiety as they occur in alcoholic patients. To do this, we will examine cerebrospinal fluid and plasma for norepinephrine (lying and standing), urine for catecholamine metabolites and employ pharmacological challenge paradigms using lactate, isoproterenol and chlorimipramine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 0267-05 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Eckardt	Section Chief	LCS, NIAAA
Others:	E. Joyce	Visiting Associate	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA
	R. Momenan	Visiting Fellow	LCS, NIAAA
	R. Rawlings	Mathematical Statistician	DBE, NIAAA
	D. Rio	Physicist	LCS, NIAAA
	J. Rohrbaugh	Research Psychologist	LCS, NIAAA
	U. Ruttimann	Biomedical Engineer	DSB, NIDR

COOPERATING UNITS *(if any)*

Radiation Oncology Branch, NCI (E. Lamoreau)

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Laboratory of Clinical Studies

SECTION

Section of Clinical Brain Research

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various clinical imaging methods are being used to study the brain in vivo. These techniques enable comparisons of gross anatomy (CAT-Computed Axial Tomography' MRI - Magnetic Resonance Imaging) of the brain with electrical activity (EEG - electroencephalography; ERPs-Event-Related Potentials) and rate of glucose utilization in a specific regions (PET - Positron Emission Tomography). From a clinical perspective, these techniques, in association with other diagnostic tests, enable qualitative judgments to be made as to the anatomic and physiologic integrity of the brain. In order to quantitatively analyze image data, the imaging techniques themselves are being investigated, as well as the effects of the associated mathematical models and subjective inputs on the reconstruction of the brain image. Moreover, mathematical and statistical methods for evaluating and relating these various sources of multivariate data are being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00268-05 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Effects of Ethanol and other Psychotropic Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Lister	Visiting Scientist	LCS, NIAAA
Others:	M. Durcan	Visiting Associate	LCS, NIAAA
	M. Eckardt	Section Chief	LCS, NIAAA
	D. Goldman	Section Chief	LCS, NIAAA
	L. Hilakivi	Visiting Fellow	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

George Washington Univ. (H. Weingartner); VA Medical Center, Portland, OR (J. Crabbe); United States Soldiers' Airmen's Home, Washington, D.C.

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Brain Research

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pharmacologic and genetic methods are being used to determine the psychobiological mechanisms underlying various behavioral processed. The current research is focusing on the neurobiology of anxiety, implusivity and aggression in mice and on learning and memory processes in humans. The effects of ethanol, and of drugs with known and specific mechanisms of action are being investigated.

Selective alpha-2 adrenoceptor antagonists have been shown to reduce ethanol's hypothermic and ataxic effects but not to alter ethanol's anxiolytic, anticonvulsant or locomotor stimulant effects. The effect is mediated centrally rather than peripherally and appears to involve alpha-2 adrenoceptors rather than imidazoline binding sites.

Male mice that show high levels of aggression towards their cage-mates differ from both their subordinate cage-mates and from control mice housed in cages in which little fighting is observed. The most consistent difference is observed in Porsolt's swim test subordinate mice show increased immobility relative to controls. The behavioral differences appear to follow rather than precede the aggressive social interactions.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00270-05 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Impulsivity and Pathologic Gambling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Roy	Visiting Associate	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	J. DeJong	Staff Fellow	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was conducted to investigate biological substrates of pathological gambling. We found indices of increased central nervous system noradrenergic activity. Also, depressed gamblers showed evidence of abnormal glucose homeostasis. Furthermore, indices of noradrenergic activity correlated significantly with extraversion scores on the Eysenck personality questionnaire suggesting that biological abnormality in gamblers may manifest itself by an effect on personality. In a stydy of GABA we found no difference in CSF levels between gamblers and controls or between depressed and non-depressed gamblers.

This study has been completed and terminated.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00272-03 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CSF Monoamine Metabolites in Alcoholic Patients who Attempt Suicide

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Linnoila	Chief	LCS, NIAAA
Others:	D. George	Acting Section Chief	LCS, NIAAA
	J. DeJong	Staff Fellow	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Reduced cerebrospinal fluid levels of the serotonin metabolite 5-hydroxyindoleacetic acid have been reported to be commonly associated with suicidal behavior. Alcoholics are known to often manifest suicidal behavior. Therefore, we compared cerebrospinal fluid levels of monoamine metabolites among alcoholics who had (N=18) or had not (N=132 attempted suicide, and controls (N=29). There were no significant differences among the three groups for cerebrospinal fluid concentrations of either 5-hydroxyindoleacetic acid, the dopamine metabolite homovanillic acid, norepinephrine, or the norepinephrine metabolite 3-methoxy-4-hydroxpenylglycol. However, in an expanded data set of almost 300 alcoholics there were significant differences for age of onset alcoholics who attempted suicide had an early age of onset of heavy drinking. They also had significantly more lifetime psychiatric diagnoses of major depression, antisocial personality disorder, panic, phobic disorder and more family history of alcoholism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00273-02 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Serotonergic Activity on Neuroendocrine & Behavioral Measures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Section Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Clinical Studies, NIMH (D. Murphy)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Science

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several studies suggest possible serotonergic involvement in the neurobiology of alcoholism and panic disorder. To evaluate this possibility we administered the serotonin agonist m-chlorophenyl piperazine (m-CPP) to alcoholics, alcoholics with panic disorder and controls. By observing the drug-induced behavioral effects and measuring changes in prolactin, cortisol and ACTH we hope to make inferences about post-synaptic serotonin function in these patient populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AAA 00274-02 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intravenous Procaine in Alcoholics and Adult Children of Alcoholics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Sction Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, NIMH (R. Post); Laboratory of Psychology, NIMH (R. Coppola)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the present study procaine is administered to alcoholics, children of alcoholics and normal controls. In addition, the patient groups will be separated into those with and without panic attacks. Mood changes have been diverse, ranging from euphoria to dysphoria. Bipolar patients tended to experience more physical symptoms, while patients with borderline personality showed dysphoria both at baseline and after procaine. Procaine also increased plasma ACTH, cortisol and prolactin, but not growth hormone. Because procaine selectively stimulates the temporal lobes of the brain, these findings suggest that the mood changes may originate in this area of the brain. These studies have also shown that the procedure is safe and not excessively stressful to patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00275-02 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Psychomotor and Cognitive Aspects of Alcoholism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	M. Eckardt	Section Chief	LCS, NIAAA
Others:	D. George	Acting Section Chief	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA
	J. Rohrbaugh	Research Psychologist	LCS, NIAAA

COOPERATING UNITS *(if any)*

Georgetown University, Washington, D.C. (S. Gilson); VAMC, Charleston, S.C. (B. Adinoff)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Brain Research

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	2.2	.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This series of studies is designed to investigate the neuroanatomical and neurochemical pathways underlying impaired cognitive and psychomotor functions in detoxified adult alcoholics and their offspring. We have demonstrated that long-term alcohol abuse is associated with unusual saccadic eye movements in about half the alcoholics studied. Low doses of diazepam administered i.v. to these alcoholics reduce the number of unusual eye movements. It is anticipated that studies on the children of alcoholics will clarify whether these unusual eye movements predate the onset of excessive alcohol consumption.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00276-02 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiology and Behavior of Aggression and Suicide in Adults and Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. Name, title, laboratory, and institute affiliation)

PI:	G. Brown	Unit Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS (if any)

Office of the Administrator, ADAMHA (F. Goodwin)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects ___ (b) Human tissues ___ (c) Neither

X (a1) Minors

___ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies that relate human (including Disruptive Behavior Disorders in children) aggression and suicide to various behavioral and biological factors have been ongoing. The most significant findings a trivariate relationship among a history of aggressive behavior, a history of suicidal behavior, and low cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5HIAA). The data indicate that certain aggressive, impulsive, and depressive characteristics in childhood are inversely related to CSF 5HIAA measured during late adolescence; family instability (particularly, alcoholism in a parent) during childhood is also associated with an increased likelihood of aggressive and suicidal behavior in adolescence. Offspring of parents with psychopathology, particularly major affected disorders are more likely to manifest suicidal behavior as adolescents than offspring of control parents. These data, along with the work of other investigators studying aggressive and depressive behavior in childhood, indicate the possibility of traits associated with disordered serotonin metabolism; further, the less consistent relationship between lower CSF 5HIAA and suicidal behaviors vs. aggressive behaviors, may indicate that some suicidal behaviors are a self-destructive manifestation of a more basic destructive (aggressive/impulsive) trait.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00277-02 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Non-human primate models of alcohol consumption and aggression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. D. Higley	Staff Fellow	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	D. Goldman	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Comparative Ethology, NICHD (S. Suomi, K. Abbott)
EBON (A. Dodson)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, NIH Animal Center, Poolesville, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.4	1.4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

During the past year, three major lines of parallel research have been pursue Research concerning--(1) Continued research concerning the effect of peer-only rearing and individual differences in anxiety on alcohol consumption. To replicate our original findings of peer-reared induced alcohol consumption additional peer-reared subjects were studied. Links between individual differences in anxiety and alcohol consumption were also investigated. (2) Studies of ferally living monkeys: To assess the generalizability of our laboratory findings, and to obtain a subject pool with sufficient numbers of inappropriately agressive individuals to perform parametric analyses, a study of ferally-living drawn from a subject pool of 4,000 rhesus monkeys off the coast of South Carolina was initiated. (3) Treatment of high alcohol consumption in peer-only reared monkeys: A number of studies indicate a relationship between diminished serotonin turnover and increased alcohol consumption. A study was completed to administer sertraline, a potent serotonin reuptake blocker to alcohol consuming monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 0278-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral and Physiological Effects of 2-Deoxyglucose Infusions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Section Chief	LCS, NIAAA
Others:	T. Alim	Staff Fellow	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

CNSB, NIMH (D. Pickar)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

2-deoxyglucose (2-DG) is a glucose analog which competitively inhibits glucose-6-phosphate dehydrogenase and leads to intracellular glucoprivation. In previous studies, 2-DG has been used as a stressor to activate both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenal axis and stimulate the appetitive centers of the hypothalamus. Our interest in this paradigm was generated by our clinical observation that alcoholics frequently consume increased amounts of carbohydrates following cessation of drinking. In order to explore possible hypothalamic abnormalities in subjects with alcoholism, we administered 2-DG to abstinent alcoholics that were classified into Type I and Type II subgroups using von Knorring's criteria (2). In this study we explored the behavioral and physiological changes arising from the 2-DG challenge. We postulated that a 2-DG induced glucoprivic response would give rise to both neuroendocrine and behavioral changes that might elucidate mechanisms of alcohol's action in the hypothalamus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AA 00279-01 LCS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Black and White Offspring of Parental Alcoholics versus Control Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	V. Moore	Social Worker	LCS, NIAAA
Others:	G. Brown	Unit Chief	LCS, NIAAA
	M. Linnoila	Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Clinical Studies

SECTION
Section of Clinical Science, Unit of Family Studies

INSTITUTE AND LOCATION
NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.35	.30	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study compares Black and White adult offspring from middle class families with parental alcoholism (ACOA - adult children of alcoholics) and without parental alcoholism (ACONA - adult children of non-alcoholics) with age similar sex and race matched controls. Preliminary findings include a greater frequency of both alcoholism and major depression in ACOA when compared with ACONA. Males appear to be at higher risk for alcoholism while females are at higher risk for major depression. No racial differences are apparent except for a possible increased risk of minor depression in black women among both ACOA and ACONA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00280-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Studies of the Electroencephalogram and Event-Related Potentials

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Goldman	Section Chief	LCS, NIAAA
Others:	M. Eckardt	Section Chief	LCS, NIAAA
	M. Enoch	Visiting Fellow	LCS, NIAAA
	J. Rohrbaugh	Research Psychologist	LCS, NIAAA
	L. Brown	Unit Chief	LCS, NIAAA
	V. Moore	Social Worker	LCS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Viral Carcinogenesis, NCI (S. O'Brien), Program Resources, Inc., Frederick, MD (M. Dean), LPPS, NIAAA (P. Hoffman).

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section on Genetic Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	2	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A path for mapping genes for alcoholism is to identify related traits which exhibit defined patterns of Mendelian inheritance. We are identifying families with EEG and event-related potential (ERP) variants and studying the transmission, relationship to alcoholism and other behaviors, and genetic linkage of these variants. In a genetic epidemiological study, the low voltage (LV) alpha EEG variant was confirmed to be transmitted in autosomal dominant fashion. However, the monomorphic alpha resting EEG pattern was found to be largely sporadic in its distribution. Alcoholism, depression and anxiety were increased in individuals with the LV trait. Six families with the LV variant are being analyzed for genetic linkage to dispersed DNA markers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00281-01 LCS

PERIOD COVERED

October 1, 1989 to September 30. 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Studies on Alcoholism in American Indians

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Goldman	Section Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	A. Bolos	Visiting Fellow	LCS, NIAAA
	G. Brown	Unit Chief	LCS, NIAAA

COOPERATING UNITS (if any)

Laboratory of Viral Carcinogenesis, NCI (S. O'Brien); Program Resources Inc., Frederick, MD (M. Dean); Indian Health Service, Clinton, OK (B. Albaugh); University of New Mexico (J. Long, F. Romero); Hopi Found., Sec, Mesa, AZ

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section on Generic Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To identify alcoholism risk genes, we are collecting and testing for linkage families from Indian populations which are relatively homogeneous and in which alcoholism is highly prevalent. This study also addresses the genetic epidemiology and psychiatric comorbidity of alcoholism in Indians. Studies are underway at three sites. Two hundred Cheyenne Indians have been clinically evaluated and their cell lines immortalized. Genetic linkage is underway on this group. At Jemez Pueblo, data and cell lines have been collected for 100 subjects. A study on the Pima Indians is in progress. Frequency of the inactive ALDH and of flushing is low in Cheyenne Indians, as was previously shown by Li et al. for Southwest Pueblo Indians. A synthesis of available population and molecular genetic data indicates a probable role for a selective force (such as a disease) in maintaining the inactive ALDH variant at high frequency in Oriental and South American Indian populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00282-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Studies on the Dopamine D2 Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	D. Goldman	Section Chief	LCS, NIAAA
Others:	A. Bolos	Visiting Fellow	LCS, NIAAA
	G. Brown	Unit Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

Program Resources Incorporated, Frederick, MD (M.Dean)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section on Genetic Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

A highly significant population genetic association had been reported between alcoholism and a TaqI DNA polymorphism recognized by the dopamine D2 receptor gene probe (source: D.Grandy). We tested this linkage using two D2 DNA markers in a population of 40 well-characterized alcoholics and a control population and in two informative pedigrees. The second DNA marker was a novel one, generated by the technique of polymerase chain reaction (PCR) followed by nondenaturing DNA electrophoresis of single-stranded DNA to detect a conformational polymorphism (SSCP). The dopamine D2 receptor gene and alcoholism were shown not to be strongly linked in the general population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00283-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Studies on Enzymes of Alcohol Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Goldman	Section Chief	LCS, NIAAA
Others:	T. Moretti	Predoctoral IRTA	LCS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Viral Carcinogenesis, NCI (S. O'Brien), PRI, Frederick (M. Dean); Univ. of Indiana School of Medicine (H. Edenberg); LPPS, NIAAA (P. Rathnagiri); NIAID, NIH (C. Kozak)

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Genetic Studies

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Among the candidate genetic loci for alcoholism and for differences in alcohol's toxic effects are genes encoding alcohol metabolic enzymes. Class III ADH (ADH5) is an unusual ADH which has an important role in the metabolism of formaldehyde (Koivwasalo, 1990). This enzyme could thus play a role in methanol toxicity and in the metabolism of methanol found as a trace contaminant in alcoholic beverages. We have shown that this is the only ADH present in significant amounts in brain. We cloned the human class III alcohol dehydrogenase and determined that it was highly distinct in sequence as compared to the previously known ADHs. The three ADH classes were approximately equal in their sequence identities to each other. Analysis of tissue mRNA levels revealed that class III ADH, unlike the other ADHs, was expressed in all tissues. A moderately informative human Sac I RFLP was discovered. We located the gene to the same chromosomal region in mouse and man, approximately 5 cM from the other ADH genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00284-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alterations in Lipid Metabolism in the Nervous System by Ethanol

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.Y. Kim	Senior Staff Fellow	LCS, NIAAA
Others:	S. Sawazaki	Visiting Fellow	LCS, NIAAA
	N. Salem	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Analytical Chemistry

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20829

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal objective of this study is to elucidate metabolic functions of polyunsaturated fatty acids and phospholipids in nervous tissues with particular reference to their modulation by ethanol. Our studies focused on the major polyunsaturate of brain, docosahexaenoic acid (22:6n3) and to a lesser extent, on arachidonic acid (20:4n6). Two aspects were studied: i) oxygenation of polyunsaturates in brain. ii) non-oxidative metabolism of polyunsaturated fatty acids to the corresponding ethyl esters.

Progress has been made in characterizing hydroxy forms of both 22:6n3 and 20:4n6 formed by rat brain with the aid of reference compounds which were prepared by autooxidation or reaction with soybean, potato or platelet lipoxygenases. Based upon the inhibitor profile and their stereochemical purity it is suggested that peroxidation but not lipoxygenation was the major mechanism of their formation in brain parenchyma. Brain microvessels showed 12-lipoxygenase activity although the level of production was low. During the course of this study, new techniques were developed in analyzing lipoxygenase and autooxidized products: i) Thermospray LC/MS analysis of hydroperoxy-derivatives provided information regarding the position of oxygenation. ii) Thermospray analysis of leukotrienes in negative ion mode as pentafluroßenzyl derivatives provided a sensitive tehcnique for measurement of the level of leukotrienes in liological samples. iii) A chiral phase HPLC system was established for analysis of the stereochemical distribution of hydroxy derivatives for various polyunsaturated fatty acids.

Ethyl ester formation was examined in rat brain in the presence of alcohol primarily from 22:6n3 since this fatty acid is highly localized in brain tissue. As has been shown for other fatty acids, 22:6n3 was transformed to the ethyl ester form at a physiological concentration of ethanol. The amount of this production correlated with the ethanol concentration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00285-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Functions of Lipoxygenase Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Karanian	Research Physiologist	LCS, NIAAA
Others:	H-Y. Kim	Senior Staff Fellow	LCS, NIAAA
	N. Salem	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Analytical Chemistry

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The n-3 fatty acids, eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3), are known to be lipoxygenase substrates. We have previously reported that mammalian platelets enzymatically produce 12(S)-hydroxy-eicosapentaenoate (12-HEPE) and 14(S)-hydroxydocosahexaenoate (14-HDHE) from an exogenous source of fatty acids. Our present studies have investigated (i) the effects of ethanol and dietary permutations on the production of eicosanoids and docosanoids and (ii) the biological activities of these metabolites in platelet-smooth muscle cell interactions as well as other cellular responses.

Dietary supplementation with n-3 fatty acids leads to the replacement of 20:4n6 with 20:5n3 and 22:6n3 in rat platelet. The eicosanoid/docosanoid profile was shifted towards the n-3 products as reflected by an increase in platelet 12-lipoxygenase products of 20:5n3 (12-HEPE) and 22:6n3 (14-HDHE) and decreased 20:4n6 products compared to an n-6 supplemented group. Platelets obtained from ethanol-dependent rats produce markedly more HDHE following up to a 7 day exposure, however, no change in HDHE production was observed following a 14 day ethanol exposure. The hydroxylated fatty acids (HEPE and HDHE) have been biosynthesized and purified in order to examine their functions in smooth muscle. It was confirmed that they antagonize thromboxane-induced contractile responses. This effect was most significant in vascular smooth muscle in comparison

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00286-01 LCS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychobiology of Alcoholism in Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. George	Acting Section Chief	LCS, NIAAA
Others:	M. Linnoila	Chief	LCS, NIAAA
	T. Alim	Clinical Associate	LCS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Studies

SECTION

Section of Clinical Science

INSTITUTE AND LOCATION

NIAAA, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies suggest the pathophysiology of alcoholism may differ significantly between men and women. To explore this possibility we have employed several pharmacological challenge paradigms to compare the behavioral and endocrine responses between male and female alcoholics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00400-05 USP

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Selective Breeding for Ethanol Tolerance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.A. Grant	Staff Fellow	USP, NIAAA
	B. Tabakoff	Scientific Director	NIAAA
Other:	P.L. Hoffman	Section Chief	LPPS, NIAAA

COOPERATING UNITS *(if any)*

VRB, DRS (C. Hansen); BIOQUAL (B. Till, T. Calzone)

LAB/BRANCH

Unit for Special Projects

SECTION

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	0.8	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This experiment is a continuing project examining if tolerance to ethanol following chronic exposure has a large genetic influence. Over the past year, 2 selected generations of both replicate lines (400 rats in total) have been tested and bred for the degree of tolerance to ethanol. Presently, the third generation for two lines have been tested (100 rats). Based on the results of previous studies using an ethanol related trait as the phenotype, selected lines do not clearly start to separate until the fourth or fifth generation. Thus, this project is still in the early stages, and an evaluation of the success of the project is premature. However, there does appear to be a decrease in the amount of variance in the degree of tolerance acquired across the three generations tested as well as a tolerance to length of time the animals have lost their righting reflex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00401-03 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interaction Between the Immune System and Adrenergic Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

P.I.:	G. Kunos	Laboratory Chief	LPPS, NIAAA
Others:	T. Szentendrei	Visiting Fellow	LPPS, NIAAA
	T. Nakane	Visiting Fellow	LPPS, NIAAA
	M. Virmani	Research Chemist	LPPS, NIAAA
	E. Lazar-Wesley	Senior Staff Fellow	LPPS, NIAAA
	L. Takacs	Visiting Associate	LPPS, NIAAA

COOPERATING UNITS *(if any)*

SUNY, Stonybrook, NY (C.C. Malbon)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.0	3.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The results of a study on the interaction of cytokines with beta-adrenergic receptors (betaAR) in cultured human lung tumor cells indicate that the density of betaAR, assayed by ^{125}I-pindolol binding, is increased 2-3-fold by a 24 hr incubation of A549 cells with IL-1alpha, IL-1beta, and TNFalpha (EC50: 2.7, 8.2 and 24 pM, respectively), while a series of other cytokines do not have this effect. Cortisol also increased betaAR density (EC50: 30 nM), and markedly potentiated the effects of IL-1alpha, IL-1beta, and TNFalpha. Neither IL-1 nor cortisol influenced the proportion of cell surface vs. internalized betaAR. The IL-1-induced increase in betaAR density was half-maximal after 6 hr, was reversible at a similar rate, and was blocked by 1 μM of cycloheximide. The effect of IL-1 on betaAR was specific, as the density of glucocorticoid receptors, measured by ^{3}H-dexamethasone binding, was reduced by IL-1. Both cortisol and IL-1 potentiated the isoproterenol-induced increase in cAMP accumulation. IL-1 inhibited cell proliferation and thymidine uptake, and increased the adherence of A549 cells to the plastic culture flask, as quantified by a cell detachment assay. The effect of IL-1 on cell adherence was not inhibited by cycloheximide. Cortisol decreased cell adherence and prevented the IL-1-induced increase in adherence. The results indicate that multiple effects of IL-1 in a cultured tumor cell line involve different mechanisms, suggesting heterogeneity of IL-1 receptors or coupling of the IL-1 receptor to distinct, nuclear and nonnuclear, effector pathways. Earlier studies indicated that IL-1 selectively upregulated the beta2AR subtype. Ongoing experiments are aimed to determine the effects of IL-1 at the level of the transcription of the beta2AR and beta1AR genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AA 00402-03 LPPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brainstem Neuromechanisms in Blood Pressure Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G. Kunos	Laboratory Chief	LPPS, NIAAA
Others:	A. Florentino	Special Volunteer	LPPS, NIAAA
	J.A. Mastrianni	Staff Fellow	LPPS, NIAAA
	K. Varga	Visiting Fellow	LPPS, NIAAA

COOPERATING UNITS *(if any)*

LCB, NIMH (M. Palkovits)

LAB/BRANCH
Laboratroy of Physiologic and Pharmacologic Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIAAA, 12501 Washington Avenue, Rockville MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	1.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ethanol has important cardiovascular effects, including inhibition of the depressor baroreflex response. GABA is the main inhibitory neurotransmitter in the CNS, and hypothalamic GABAergic neurons exert powerful inhibition of the baroreflex at the level of the nucleus tractus solitarii (NTS). As the first step toward our long range goal to clarify the effects of ethanol on cardiovascular regulation, we examined the mechanism of the pressor response of the GABA-B agonist baclofen at the level of the NTS. In urethane-anesthetized rats, intra-NTS baclofen caused dose-dependent pressor effects and tachycardia and inhibited baroreflex bradycardia elicited by iv. phenylephrine. The effects of baclofen were inhibited by intra-NTS phaclofen and 2-OH-saclofen, as well as by the non-NMDA glutamate antagonist DNQX and by ic. pretreatment with pertussis toxin (PT). DNQX and PT, but not the NMDA antagonist MK-801, also inhibited baroreflex bradycardia. Intra-NTS glutamate caused hypotension and bradycardia, which were potentiated by baclofen, and not inhibited by either DNQX, MK-801 or PT. These findings indicate that the cardiovascular effects GABA-B receptor stimulation in the NTS are due, at least in part, to inhibition of the depressor baroreflex response. Inhibition of the release and/or action of an excitatory amino acid other than glutamate is the most likely mechanism.

In a subsequent study we found that the GABA-A agonist, muscimol, also causes pressor effects and inhibits baroreflex bradycardia, when injected into the NTS. Iv. or intra-NTS ethanol also inhibited baroreflex bradycardia and potentiated the effects of muscimol. The baroreflex inhibitory action of ethanol was eliminated by depletion of GABA by 3-mercaptopropionate, and reduced by bicuculline or by RO-15-4513. These findings suggest that ethanol inhibits the baroreflex partly through potentiation of the effects of endogenous GABA on GABA-A receptors in the NTS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AA 00403-03 LPPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inverse Regulation of Hepatic alphal and beta-adrenergic Receptors.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G. Kunos	Laboratory Chief	LPPS, NIAAA
Others:	E. Ishac	Visiting Associate	LPPS, NIAAA
	E. Lazar-Wesley	Senior Staff Fellow	LPPS, NIAAA
	M. Grojec	Visiting Associate	LPPS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Physiologic and Pharmacologic Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The fatty acid composition of total lipids and the adrenoceptor mediated activation of glycogenolysis were studied in isolated hepatocytes from rats maintained on a control diet or on an essential fatty acid (EFA)-free diet. In cells from rats on the EFA-free diet there was a marked reduction in linoleic and arachidonic acid (AA) contents and an increase in eicosatrienoic, oleic and palmitoleic acid contents compared to controls. In freshly isolated cells from both groups, the adrenergic activation of glycogen phosphorylase was mediated only by alphal and not by beta-receptors. When control cells were preincubated in a serum-free buffer for 4 hours before testing, the alpha-adrenergic component was blunted and a cAMP dependent beta-adrenergic component of the phosphorylase response emerged. A similar 4 hr incubation of EFA-deficient cells resulted in a reduced alpha but continued absence of a beta-receptor response. A beta-receptor response of these 4 hr cells could be restored by in vivo replacement of the EFA-deficient diet with control diet for the last 4 weeks prior to the experiment, but not by the in vitro exposure of EFA-deficient cells to 10 uM AA throughout the 4 hr incubation period. Extending previous observations, the present results suggest that the time-depndent emergence of beta-adrenergic glycogenolysis, but not the parallel reduction of the alpha-adrenergic response, is mediated by AA or its metabolite(s), which probably act by facilitating the G-protein dependent coupling of beta-receptors.

Inverse regulation of hepatic alphal and beta2 receptors has been demonstrated in rat liver cells in primary culture DNA solution hybridization assays for alphal and beta2 receptors have been developed to analyze the transcriptional regulation of the two receptors in the above experimental model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00404-03

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of calcium- and phosphorylation-regulated signaling pathways

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.L. Kincaid	Section Chief	LPPS, NIAAA
Others:	T.M. Martensen	Research Chemist	LPPS, NIAAA
	J. Tamura	Visiting Fellow	LPPS, NIAAA
	S. Higuchi	Visiting Fellow	LPPS, NIAAA
	S.C. Dixon	Microbiologist	LPPS, NIAAA
	C.A. Marietta	Research Physiologist	LPPS, NIAAA
	P.R. Giri	Visiting Associate	LPPS, NIAAA

COOPERATING UNITS *(if any)* Penn State Univ. (M.L. Billingsley, C.D. Balaban); Lab. of Immunology, NIAID, NIH (M. Sitkovsky); Univ. of Illinois, Peoria (N. Ludvig); Columbia University (R.H. Kessin); Molec. Neurogenetics Branch, NIMH, ADAMHA, (B.M. Martin)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section on Immunology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.8	3.4	.04

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of mammalian cDNA and genomic clones for the catalytic subunit of th calmodulin (CaM)-dependent protein phosphatase, calcineurin (CN) indicat multiple molecular isoforms that result from alternative splicing of distinc genes. The evolutionary relationship between these genes has been characterized as have their localizations on human chromosomes and differences in mRN expression. To study the origins of this genetic complexity and its regulator controls, we are comparing the corresponding genes in lower eukaryotes. In thes simpler organisms primary structure is highly conserved in catalytic an regulatory domains, while regions of unrelated sequence near the carboxy terminus suggest areas that may impart unique substrate specificity. I Neurospora intron/exon boundaries corresponded to those we have found in th mammalian gene, suggesting a step-wise evolution of DNA control features. In bot Neurospora and Dictyostelium, mRNA expression appears to be developmentall controlled, and studies to examine promoter regulation are being pursued. bacterially-expressed fungal catalytic subunit having high enzyme activity wa produced and a set of deletion mutants of the mammalian protein delineated th minimum structural domain needed for CaM binding; a synthetic peptide based o this region exhibited an inhibitory constant of ½5 nM. Developmental studies o phosphodiesterase (PDE) showed a pattern of expression that coincided wit synaptogenesis in most brain areas; the e.m. localization of PDE in neurons i now being studied to suggest its possible functional roles. A novel brain specific CaM binding protein of 140 kDa (native MW) has been characterized tha is localized primarily in limbic structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00405-03 L

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection and regulation of specific cellular phosphoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.M. Martensen	Research Chemist	LPPS, NIAAA
Others:	R.L. Kincaid	Section Chief	LPPS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Molecular Neurogenetics, NIMH (B. Martin)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section of Immunology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phosphorylation of a Calmodulin (CaM)-dependent phosphoprotein phosphatase, calcineurin, (CN) by a CaM-dependent protein kinase modifies a specific serin residue. Dephosphorylation occurs by a mechanism in which the catalytic site of a modified polypeptide hydrolyses its own phosphoserine bond. Thi autodephosphorylation can be regulated by effectors of CN enymatic activity Activators of the reaction were cations of Mn, Ca, and Ni. Ca\CaM, whic stimulates intermolecular phosphatase activity, inhibits autodephosphorylatic presumably by binding to a primary sequence of CN juxtaposed with th phosphoserine. Certain inhibitors of CN activated autodephosphorylation such a phenothiazine drugs (trifluoperazine, and chlorpromazine) and metal ion chelator (EGTA, and EDTA). In contrast to the finding of others who found tha phosphorylation inhibited the dephosphorylation of certain substrates, th phosphorylated enzyme dephosphorylated Inhibitor-1 at a more rapid rate than th dephosphorylated CN. A soluble enzymatic activity in brain exists that rapidl dephosphorylates phosphoCN. This activity is Ca\CaM independent and inhibited b nM concentrations of okadaic acid which is a characteristic of phosphoprotei phosphatase 2A. The ability to dephosphorylate phosphoCN is however dependen on denaturation of phosphoCN and maintenance of a low level of sodium dodecy sulfate in the reactions. These features suggest the phosphoserine residu exists in a constrained structure which requires conformation change for facil dephosphorylation to occur. Antibodies obtained from rabbits immunized wit synthetic peptides composed of conserved sequences found in CN and othe phosphoprotein phosphatases bound radiolabeled CN. Antibodies affinity purifie on peptide-Sepharose columns were incubated with radiolabeled CN and adsorbed t protein-A Sepharose. Bound CN was eluted by denaturating conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00406-01 LPPS

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Noradrenergic Neurotransmission and Actions of Ethanol

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E. Ishac	Visiting Associate	LPPS, NIAAA
Others:	G. Kunos	Laboratory Chief	LPPS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotransmitter release is triggered by an elevated level of free calcium and thus represents a crucial event in synaptic transmission. However the biochemical processes involved in calcium influx and calcium-dependent transmitter release are not known. Ethanol can alter the mobilization of calcium in a number of cells systems including synaptic transmission. The calcium/phospholipid-dependent protein kinase, protein kinase C, is highly localized in neuronal tissue and in particular presynaptic nerve terminals. I have examined the role of activation or inhibition of protein kinase C on the release of noradrenaline from rat isolated atria preloaded with [3-H]-noradrenaline. It was found that activation of protein kinase C by phorbol 12-myristate 13-acetate caused a concentration-dependent enhancement of membrane depolarization induced (electrical field stimulation or high potassium) release of noradrenaline. Whereas polymyxin B, an inhibitor of protein kinase C reduced noradrenaline release evoked by either electrical field stimulation or high potassium. In contrast, non-exocytotic release of noradrenaline evoked by tyramine was not altered by phorbol 12-myristrate 13-acetate. Polymyxin B only at a high concentration caused a slight reduction in tyramine-induced outflow of radioactivity. The findings suggest that protein kinase C may play a role in the exocytotic release of noradrenaline but not due to displacement. Ongoing studies will examine the effect of acute and chronic ethanol treatment on the calcium/protein kinase systems involved in neurotransmission.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00479-07 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synaptic Mechanisms and Ethanol Actions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	F.F. Weight	Section Chief	LPPS, NIAAA
Others:	D.M. Lovinger	Staff Fellow	LPPS, NIAAA
	R.W. Peoples	NRC Fellow	LPPS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section of Electrophysiology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.7	2.2	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms underlying the behavioral effects of alcohol have not been established. This project studied the cellular and molecular actions of ethanol on neurotransmitter-activated membrane ion channels in mammalian neurons using the whole-cell patch-clamp recording technique. The ion current activated by the glutamate agonist NMDA was inhibited by ethanol; the inhibition increased in a concentration-dependent manner over the concentration range 5 - 50 mM, a range that produces intoxication. This concentration range had relatively little effect on the ion currents activated by the glutamate agonists kainate and quisqualate; however, these currents were significantly inhibited by ethanol in a concentration-dependent manner over the concentration range 50 - 100 mM, a range that produces general anesthesia. Similar effects of ethanol were observed on NMDA and non-NMDA glutamate receptor-mediated excitatory postsynaptic potentials (EPSPs). The potency for inhibition of the NMDA-activated current by several alcohols was linearly related to their intoxicating potency, suggesting that the alcohol-induced inhibition of responses to NMDA receptor activation may contribute to the neural and cognitive impairments associated with intoxication. GABA-A activated ion current was potentiated by ethanol in some neurons and not affected by ethanol in other neurons. The concentration range that potentiated GABA-A activated current was 10-50 mM. GABA-A activated current was also potentiated by benzodiazepines, suggesting that the augmentation of this current by ethanol may contribute to the anxiolytic actions of ethanol. Intoxicating concentrations of ethanol were also found to potentiate the ion current mediated by activation of the 5-HT-3 type of serotonin receptor. Behavioral experiments suggest that this effect may be associated with the recognition of ethanol action. The preceding observations are consistent with the idea that the behavioral effects of ethanol may result from actions of ethanol on these receptor-gated ion channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00480-07 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nerve Cell Excitability and Ethanol Actions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: F.F. Weight	Section Chief	LPPS, NIAAA
Others: L.G. Aguayo	Senior Staff Fellow	LPPS, NIAAA
G.G. White	Staff Fellow	LPPS, NIAAA

COOPERATING UNITS *(if any)*

Dept. of Pharmacology, Univ. Pittsburgh Sch. Med. (W.D. de Groat)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section of Electrophysiology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although alcohol is known to affect the excitability of the nervous system, the mechanisms involved in those actions are poorly understood. This project studied the cellular and molecular mechanisms regulating nerve cell excitability and the effects of ethanol on those mechanisms. The membrane ion channels that are involved in the intrinsic regulation of neuronal excitability were investigated using the whole-cell patch-clamp technique. In neurons that exhibit burst-firing behavior, generation of an action potential elicited a large depolarizing afterpotential that triggered the firing of a burst of action potentials. Voltage-clamp analysis revealed that the depolarizing afterpotential was generated by a large transient (T-type) calcium current. The burst-firing behavior developed postnatally, in contrast to the long-lasting (L-type) calcium current which did not change significantly in amplitude during development. To identify the types of isolated neurons that exhibit various patterns of firing behavior, the fluorescent dyes fast blue and fluorogold were injected in the distribution field of various types of axons and time allowed for retrograde transport. The dyes were retained after isolation of the neuronal soma, permitting identification of various neuronal types. The regulation of excitability was studied by culturing adult mammalian sensory neurons in a medium free of serum and added growth-factors. Under these conditions, the neurons retained electrical excitability and voltage-activated sodium current was tetrodotoxin (TTX)-sensitive in all neurons tested. By contrast, freshly isolated neurons and neurons cultured in the presence of nerve growth factor (NGF) exhibited TTX-resistant sodium currents in some neurons and TTX-sensitive sodium currents in others. The results suggest that NGF may regulate the expression of TTX-resistant sodium channels. Ethanol was tested on several voltage-activated ion channels and found to have little or no effect in concentrations less than 100 mM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AA 00481-01 LPPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Physiological Regulation of IL-1 Production

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	L. Takacs	Visiting Associate	LPPS, NIAAA
Others:	L. Bristol	IRTA Fellow	LPPS, NIAAA

COOPERATING UNITS *(if any)* LCB DCTB, NCI (E. Appella); LCB DCTB, NCI (S. Moore); LMI, BRMP, NCI (S. Durum); LVC, NCI (N. Yuhki); Dept. of Surgery MGH, Harvard Med. Sch. (M. Mester)

LAB/BRANCH
Laboratory of Physiologic and Pharmacologic Studies

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

IL-1 is a major inflammatory cytokine that has diverse effects on different tissues. Despite the large number of publications on the effects of this cytokine very little is known about the physiological regulation of IL-1 gene expression and production.

The objective of this project is to analyze these regulatory factors. Three models were used.

1. We previously identified and characterized a novel T-cell derived cytokine that induces IL-1 production. We are in the process of the cloning of the cDNA.
2. Macrophages in the lamina propria of the gut express IL-1 genes in normal mice. We analyzed the factors that regulate this IL-1 expression and found that enviromental factors might be crucial in the induction of IL-1 genes in the gut macrophages.
3. Several observations suggest that IL-1 has effects on the CNS. However, we did not find IL-1 mRNA in the normal brain by a sensisitve PCR analysis. Intracerebral injection of LPS dramatically incresed IL-1 gene expression in the brain within 3 hrs.

These observations suggest that IL-1 has a role in adapting both the CNS and the immune system to the state of inflammation and immune response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00482-01 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Regulation of Early Steps in T-cell Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	L. Takacs	Visiting Associate	LPPS, NIAAA
Other:	L. Bristol	IRTA Fellow	LPPS, NIAAA

COOPERATING UNITS *(if any)*

Immunology Branch, NCI, NIH (A. Weissman); Dept. of Immunology, ELTE, Hungary (E. Rajnavolgyi)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The mechanism of proliferation of early intrathymic precursor cells is unknown. We analysed this problem by two approaches.

A: the possible involvement of several cytokines were investigated.

B: monoclonal antibodies were generated that react with T-cell precursors and influence their proliferation.

Both IL-1 and IL-2 are able to induce differentiation and growth of gamma/delta cells from CD4-/CD8- (DN) precursor cells. IL-2 induces the transcription of the TcR gamma chain gene when added to IL-1 plus mitogen cultured T-cell precursors. However, this does not result in an increase of surface TcR gamma/delta/CD3 complex expression. The results suggest that IL-2 may be involved in a critical step of development of gamma/delta T-cells, the induction of the TcR gamma chain gene.

The 1.3.1 monoclonal antibody reacts with T-cells and several other cells and tissues. Biochemical analysis of the antigen and its tissue distribution suggest that the antibody recognizes a neutral amino peptidase on the cell surface.

When added to DN cell cultures in the presence of IL-2 and PMA 1.3.1 stimulates thymidine incorporation (60%). We plan to purify the 1.3.1 antigen, to measure its peptidase activity and to determine its N-terminal amino acid sequence to answer the questions whether the antibody recognizes an aminopeptidase and whether the antigen identified is a novel protein with unknown structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00483-01 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of G Protein Coupled Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C.M. Fraser	Section Chief	LPPS, NIAAA
Others:	C-D. Wang	Visiting Associate	LPPS, NIAAA
	M.A. Buck	IRTA Fellow	LPPS, NIAAA
	N.H. Lee	IRTA Fellow	LPPS, NIAAA
	T.M. Savarese	Special Volunteer	LPPS, NIAAA

COOPERATING UNITS *(if any)*

University of Geneva (J-P. Giacobino)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section on Molecular Neurobiology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotransmitter receptors which mediate their cellular actions through guanine nucleotide binding proteins (G proteins) comprise a large gene superfamily and modulate the activity of a variety of important physiological functions. To better understand the molecular mechanisms of signal transduction mediated by this family of receptors we have utilized gene cloning, permanent gene expression in cultured cells, and site-directed mutagenesis to study the relationship between receptor structure and function and the mechanisms of receptor regulation. Using transfected cell systems expressing homogeneous populations of a single receptor subtype we have demonstrated that G protein-linked receptors are capable of activating multiple independent intracellular signalling pathways and the magnitude of cellular responses to neurotransmitters correlate with the density of receptors present in the cell membrane. These findings have important implications in understanding cellular responsiveness to hormones and neurotransmitters in vivo. Site-directed mutagenesis has allowed for the identification of conserved aspartic acid, cysteine and serine residues in adrenergic and muscarinic acetylcholine receptors which are critical for ligand biding and receptor activation by agonists. These studies have provided evidence that ligand biding to G protein-linked receptor occurs within a pocket formed by the transmembrane helices; that receptors classes which bind amine ligands share common sites for ligand recognition and that agonist activation mechanisms may involve charge transfer across the cell membrane. Finally it is known that beta-adrenergic receptors are subject to heterologous regulation by hormones such as glucocorticoids and thyroid hormones. Using receptor-specific gene probes we have shown that thyroid hormone regulation of $beta_1$- and $beta_2$-adrenergic receptor density in vivo occurs at the level of gene transcription in a tissue-specific manner. Current work is directed toward elucidating more completely the ligand binding sites of adrenergic and muscarinic acetylcholine receptors and the molecular mechanisms involved in receptor desensitization by agonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00484-01 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Expression of Ligand-gated Ion Channels in Xeonpus oocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	C.M. Fraser	Section Chief	LPPS, NIAAA
Others:	L. Aguayo	Staff Fellow	LPPS, NIAAA
	E.F. Kirkness	Visiting Associate	LPPS, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Neurotransmission in the central nervous system is mediated in large part by a group of amino acids that act via specific receptors. Ethanol, at concentrations less than 100 Mm, has been shown to affect the functional properties of two amino acid receptor systems (see Project No. AA00479-07): the $GABA_A$ receptor-activated chloride current in mouse hippocampal neurons is potentiated by ethanol and the NMDA-type glutamate receptor-activated ion current in adult DRG neurons is inhibited by ethanol. It has been suggested that the neural and cognitive impairments associated with intoxication may be related, in part, to the effects of ethanol on specific receptor systems within the CNS. In order to better understand the molecular basis for ethanol modulation of ligand-gated ion channel function and to identify the sites within these receptor systems at which ethanol may act, we have established a system for the expression of cloned receptor-channel complexes in Xenopus oocytes injected with receptor-specific MRNA. Isolated of rat brain poly (A^+) mRNA and injection into Xenopus oocytes resulted in the appearance of GABA-dependent inward currents. We are utilizing this system to study the functional properties of (1) receptors transcribed from mRNAs encoding a number of GABA receptor subtypes and (2) $GABA_A$ receptors translated from brain mRNA of long sleep and short sleep mice; and evaluating the effects of ethanol on these ion channel receptor systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA 00485-01 LPPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Structure of $GABA_A$ Receptor Subunits

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. (Name, title, laboratory, and institute affiliation)

P.I.:	E.F. Kirkness	Visiting Associate	LPPS, NIAAA
Others:	C.M. Fraser	Section Chief	LPPS, NIAAA

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NIA (J.W. Kusiak); LMCN, NINDS (J.C. Venter)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section on Molecular Neurobiology

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Ave., Rockville, MD 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major class of inhibitory neurotransmitter receptors, the $GABA_A$ receptors, are modulated by a range of depressant drugs (eg benzodiazepines, ethanol) and their disfunction has been associated with several neurological disorders. Recently, cDNA cloning has revealed the existence of numerous receptor subunit types. However, in the absence of genomic clones, it has been difficult to establish any link between inappropriate receptor expression and the implicated disease states. In addition, nothing is known of how expression of subunit genes is regulated in a coordinated and localized manner.

As an initial step towards addressing these problems, we have isolated and sequenced human genomic clones encoding $GABA_A$ receptor subunits. The complete intron/exon structure and a large region of upstream sequence was determined for the beta1 subunit gene. The beta1 subunit protein is encoded by a relatively large gene (>75 kb), comprised of nine small exons. The beta1 gene has no structural similarity to the genes encoding the nicotinic acetylcholine receptors, a related family of receptor proteins. However, exons derived from a gene encoding the beta3 subunit indicate that intron/exon boundaries are conserved precisely between subtypes of subunits. Using the genomic clones as probes, the beta1 and beta3 subunits have been assigned to chromosomes 4 and 15, respectively. Ongoing studies will use the genomic clones to probe for restriction fragment length polymorphisms (RFLPs) in families displaying inherited neurological disorders (eg alcoholism, schizophrenia). In addition, the upstream regions of the beta1 gene will be characterized in order to understand how expression of the gene is regulated in neural tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00700-06 LPPS

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ethanol Effects on Membrane-Bound Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	P.L. Hoffman	Section Chief	LPPS, NIAAA
	B. Tabakoff	Scientific Director	NIAAA
Others:	J.P. Whelan	Senior Staff Fellow	LPPS, NIAAA
	L. Karrberg	Special Volunteer	USP, NIAAA
	J. Contrera	Special Volunteer	USP, NIAAA
	E. Romm	Special Volunteer	USP, NIAAA
	D. Goldman	Section Chief	LCS, NIAAA

COOPERATING UNITS *(if any)*

University of Minnesota, Minneapolis (J.A. Halikas); Washington University School of Medicine, St. Louis (E. Devor); MDB, NIDDKD (A.M. Spiegel)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section on Receptor Mechanisms

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	2.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It has been postulated that the activities of certain enzymes may be markers of a genetic predisposition to alcoholism. We previously showed that low fluoride-stimulated platelet adenylate cyclase (AC) activity, and an increased sensitivity of platelet monoamine oxidase (MAO) to in vitro inhibition by ethanol, effectively discriminated alcoholic and non-alcoholic individuals. We have now examined the heritability of platelet fluoride-stimulated AC activity in families with alcoholic members, and have found familial transmission, with a major gene effect, for this enzyme activity. The major gene was transmitted as a Mendelian co-dominant. Transmission of basal AC activity was more complex. The findings are compatible with the possibility that fluoride-stimulated AC activity, which is transmitted as a single major gene in families of alcoholics, could represent a trait marker for a predisposition to alcoholism. AC activity was also assayed in platelets of monozygotic and dizygotic twins and concordance was higher in monozygotic twins, supporting the heritability of this measure. Platelet fluoride-stimulated AC activity is currently being compared with several other possible genetic markers of predisposition to alcoholism. Previous Western and slot blot analysis showed that the total quantity of $G_{s\alpha}$ in platelet membranes was not significantly correlated with fluoride-stimulated AC activity. Recent data indicates that there is a significant positive correlation of stimulated AC activity and cholera toxin-induced ADP-ribosylation. These results suggest that a qualitative, rather than a quantitative, difference in $G_{s\alpha}$ may contribute to lower platelet AC activity in alcoholics. The studies described will help to determine whether the observed differences in platelet enzyme activities between alcoholics and non-alcoholics are genetically based, and may be a marker for a predisposition to alcoholism, or are a consequence of ethanol consumption.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00702-06 LPPS

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Ethanol Modification of Neurotransmitter Receptor-Effector Coupling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	P.L. Hoffman	Section Chief	LPPS, NIAAA
	B. Tabakoff	Scientific Director	NIAAA
Others:	P. Valverius	Visiting Associate	LPPS, NIAAA
	J.P. Whelan	Senior Staff Fellow	LPPS, NIAAA
	K.A. Grant	Staff Fellow	USP, NIAAA
	L. Snell	NRC Fellow	LPPS, NIAAA

COOPERATING UNITS *(if any)*

Metabolic Disease Branch, NIDDKD (A.M. Spiegel); IRCM, NHLBI (J. Moss); VA Medical Center, Portland, OR (J. Crabbe); BIOQUAL (B. Till, T. Calzone)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section on Receptor Mechanisms

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.4	3.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Ethanol selectively alters the function of neurotransmitter and neuromodulator receptors in the CNS, and adaptations in receptor function may be associated with ethanol tolerance and/or physical dependence. Previous work showed decreased stimulation of adenylate cyclase by various agonists and by guanine nucleotides, and decreased high-affinity binding of a beta-adrenergic agonist and forskolin in certain brain regions of mice fed ethanol chronically. These results suggested a quantitative or qualitative change in the stimulatory guanine nucleotide binding protein, Gs. Quantitation of the two (46 and 52 kDa) forms of Gs-alpha, and of Gi-alpha, in various brain regions by Western blot analysis, however, revealed no significant change in the levels of these proteins in ethanol-fed mice, and in situ hybridization studies of Gs-alpha mRNA also showed no change. Differences in proportion of the two forms of Gs-alpha among brain regions were observed, and the 46 kDa form was found to be resistant to cholera toxin-catalyzed ADP-ribosylation. The data indicate that chronic ethanol ingestion may alter the properties rather than amount of Gs-alpha, and further characterization of the function of the two forms of the protein in ethanol-fed mice is warranted. The NMDA receptor system is also changed in ethanol-fed mice. As measured by membrane binding and autoradiographic studies of MK-801, a non-competitive antagonist at the NMDA receptor, this receptor is up-regulated in hippocampus and other brain areas of ethanol-fed mice. The time course of changes in binding parallels susceptibility to ethanol withdrawal seizures. In addition, mice bred to be prone to ethanol withdrawal seizures (WSP) have more hippocampal MK-801 binding sites than mice bred to be resistant to ethanol withdrawal seizures (WSR), both before and after chronic ethanol ingestion. These findings support a role for the NMDA receptor-gated channel in ethanol withdrawal. In preliminary studies, strychnine-insensitive glycine binding was not increased in ethanol-fed mice, suggesting a change in the function of NMDA receptor-gated channels rather than an increased number of receptor-channel

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00703-06 LPPS

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurohypophyseal Peptides and Ethanol Tolerance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.L. Hoffman	Section Chief	LPPS, NIAAA
Others:	L. Liu	Guest Researcher	USP, NIAAA
	J.R. Dave	Senior Staff Fellow	LPPS, NIAAA
	K. Gulya	Visiting Scientist	LPPS, NIAAA
	G. Szabó	Visiting Associate	LPPS, NIAAA
	K.A. Grant	Staff Fellow	USP, NIAAA

COOPERATING UNITS (if any)

VRB, DRS (C. Hansen); BIOQUAL (B. Till, T. Calzone)

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

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INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.9	3.2	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Arginine vasopressin (AVP) and related peptides, when administered exogenously, prolong the duration of tolerance to ethanol by an action at CNS V-1 receptors. Previous work suggested that stimulation of c-fos expression in the septum by AVP contributes to this action, and current structure-activity studies of peptide (AVP(4-9), nerve growth factor) effects on ethanol tolerance support this hypothesis. The finding that a V-1 receptor antagonist enhanced the rate of loss of ethanol tolerance suggested that endogenous AVP is important for maintaining tolerance. Studies with rats bred to contain the diabetes insipidus (DI) gene (which do not produce functional vasopressin) were carried out to evaluate this postulate. Rats homozygous for the DI gene acquired functional ethanol tolerance, but lost it more rapidly than normal rats or those heterozygous for the DI gene. These data led us to examine AVP synthesis during chronic ethanol treatment. In both mice and rats, hypothalamic AVP mRNA was decreased by chronic ethanol exposure. The data, particularly in rats, suggested that the effect of ethanol was most apparent in animals in which there was concomitant activation of the hypothalamo-neurohypophyseal system (e.g., dehydration). In situ hybridization also revealed that dehydration increased, and chronic ethanol ingestion decreased, vasopressin mRNA in hypothalamic areas outside the SON and PVN and in the bed nucleus of the stria terminalis, an extrahypothalamic nucleus whose neurons project to the lateral septum. These results indicate that an increase in vasopressin synthesis in brain is not necessary in order for the hormone to maintain tolerance. Understanding the mechanism by which AVP influences tolerance to ethanol may lead to benign means for the manipulation of tolerance and, possibly, of ethanol intake.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00705-04 LPPS

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Models for Ethanol Effects on Receptor-Mediated Processes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.L. Hoffman	Section Chief	LPPS, NIAAA
	B. Tabakoff	Scientific Director	NIAAA
Other:	K. Hellevuo	Visiting Fellow	USP, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Physiologic and Pharmacologic Studies

SECTION

Section on Receptor Mechanisms

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major focus of our work involves an evaluation of the acute and chronic effects of ethanol in the CNS. However, the brain represents a heterogeneous collection of cell types, and distinction of direct and indirect effects of ethanol can be difficult. In vitro cell culture systems can be used to monitor specific, direct effects of ethanol, for comparison and contrast with results obtained in brain tissue and in vivo. Current studies of the effect of ethanol on NMDA receptors in primary cultures of cerebellar granule cells are now described in project AA00706. In addition, investigations of serotonin (5HT-3) receptors in NCB20 somatic cell hybrids are being performed, and are based on the findings that 1) ethanol enhances the action of agonists at the 5HT-3 receptor in electrophysiological studies of NCB20 cells, and 2) 5HT-3 receptor antagonists block the discriminative stimulus properties of ethanol (see Project AA00707). To determine whether ethanol directly affects the interaction of ligands with the 5HT-3 receptor, antagonist and agonist binding to these receptors were assessed by filter binding techniques. The antagonist, 3H-GR65630, bound to a single site on NCB20 membranes, and agonist (5HT, 2-Me-5HT) displacement curves were best fit by a one-site model. Ethanol (12.5 - 100 mM) had no significant effect on either agonist or antagonist binding, and may instead affect receptor-effector coupling processes. The behavioral studies suggest that the action of ethanol at the 5HT-3 receptor is important in mediating subjective effects of ethanol (e.g., reinforcement, intoxication) and studies in cultured cells may suggest a biochemical mechanism underlying these in vivo responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00706-02 USP

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Effects of Ethanol on NMDA-Mediated Neuronal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	C.S. Rabe	Special Volunteer	USP, NIAAA
	B. Tabakoff	Scientific Director	NIAAA
Other:	J. Contrera	Special Volunteer	USP, NIAAA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Unit for Special Projects

SECTION

INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.75	1.25	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

One potential mechanism by which ethanol might produce its characteristic central nervous system (CNS) depression is through inhibition of excitatory transmission. Glutamate is the most abundant excitatory neurotransmitter present in the CNS. Therefore, we examined the effect of ethanol on glutamate-mediated transmission in primary cultures of cerebellar neurons. Ethanol was a potent and selective inhibitor of the actions of glutamate at the NMDA receptor subtype, and was less effective at the kainate receptor in these cells. To define the site of action of ethanol at the NMDA receptor, interactions of ethanol with several modulators of receptor function were examined. Ethanol-induced inhibition of NMDA-stimulated calcium influx was additive with inhibition produced by phencyclidine (PCP) or magnesium ion, indicating that ethanol does not act at a site within the receptor-gated ion channel. Ethanol also did not alter the EC_{50} for NMDA stimulation of calcium uptake, suggesting no direct interaction with the NMDA binding site. However, glycine, which acts at a strychnine-insensitive site to enhance responses to NMDA, and has been suggested to be a co-agonist at the NMDA receptor, was found, at high concentrations, to reverse ethanol-induced inhibition of NMDA-stimulated calcium uptake. Structure-activity studies showed that other amino acids that act as agonists at the glycine site also reversed ethanol inhibition. The data suggest that ethanol may interfere with the concerted actions of glutamate and glycine at the NMDA receptor. The specificity of ethanol's action was investigated by comparing effects of barbiturates and benzodiazepines. Barbiturates were more effective at inhibiting kainate- than NMDA-stimulated calcium influx, while flurazepam had no effect. The differential profile of drug effects at excitatory amino acid receptors may contribute to specific pharmacological actions of these drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AA00707-02 USP

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Behavioral Pharmacology of Ethanol

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.A. Grant	Staff Fellow	USP, NIAAA
	B. Tabakoff	Scientific Director	NIAAA
Other:	K. Hellevuo	Visiting Fellow	USP, NIAAA

COOPERATING UNITS *(if any)*

USUHS, Bethesda (J. Barrett)

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Unit for Special Projects

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INSTITUTE AND LOCATION

NIAAA, 12501 Washington Avenue, Rockville, Maryland 20852

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The ability of various receptor agonists and antagonists were used to characterize the discriminative stimulus effects of ethanol. Antagonists at the 5-HT3 receptor/ion complex were found to block the discriminative stimulus properties of ethanol in pigeons given orally administered ethanol. These data confirm data from electrophysiological studies which demonstrated that ethanol was potentiating the effects of serotonin at the 5-HT3 receptor. The discriminative stimulus properties of ethanol were blocked in a dose dependent manner by two antagonists acting at the 5-HT3 receptor. Increasing the dose of ethanol could overcome this blockade. The specificity of the blockade by 5-HT3 antagonists was tested using antagonists of the 5-HT2 receptor and a dopaminergic antagonist. Neither of these compounds blocked ethanol's discrimination. The ability of the 5-HT3 antagonists to block other behavioral effects of ethanol was tested using an hypnotic dose of ethanol (measuring loss of righting reflex), and a moderate dose of ethanol (measuring motor coordination). The 5-HT3 antagonists did not block either of these effects of ethanol, suggesting that the action of ethanol at the 5-HT3 receptor is not related to the motor effects of ethanol.

The role of the 5-HT3 receptor in mediating the reinforcing effects of ethanol was also tested in a preparation using a rhesus monkey self-administering ethanol intravenously.

Formerly titled "Discriminative Stimulus Effects of Ethanol."